Coevolution between pallid cuckoos *Cuculus pallidus* and their hosts

Michelle T. Landstrom

A thesis submitted for the degree of Master of Philosophy

School of Botany and Zoology
The Australian National University

May 2008

Declaration:

The research presented in this thesis is my own original work, and no part has been submitted for a previous degree. However, ideas were shared with several individuals; Dr. Naomi Langmore made important contributions in the development of the experiments and analyses, and Dr. Rob Heinsohn's work was essential in designing and understanding many of the statistical analyses.

Signed

Michelle T. Landstrom
May 2008

*Frontispiece photo taken by Julian Robinson: White-plumed honeyeater feeding a pallid cuckoo chick; Callum Brae Nature Reserve, January 2007.

Acknowledgements:

The past two years have been so unique that even two and a half years ago it would not have seemed possible. However, its fruition was meld by many remarkable individuals, many of whom I want to credit here as having guided me along my way.

My first experiences in the field and getting to understand bird observation came from Dr. Haven Wiley of the University of North Carolina at Chapel Hill. It was through his enthusiasm and appreciation of wildlife and wilderness that first cracked open the doors for me to step out of the lab and begin working in the Australian field.

Of course, this would not have been possible without my supervisor and mentor, Dr. Naomi Langmore. Her knowledge of birds and bush have helped me gain an incredible appreciation for some of Australia's greatest (yet sorely overlooked) features, and it is through her patient guidance and support that have shaped the development of this research. Thank you, Naomi, for all you have offered the past two years.

I am deeply grateful for Dr. Rob Heinsohn's help with the reflectance spectrophotometer and the analysis of its output. My statistics were certainly one of the biggest hurdles I faced, and because of Rob they became much clearer. His efforts, along with Dr. Emlyn Wilson's 'little trick', lit many paths in this seemingly endless process. Thank you also, Rob and Dr. Andrew Cockburn, for advice and comments on preliminary drafts of this study.

I spent many afternoons last autumn shuffling through museum collections across Australia, and I want to thank Dr. Leo Joseph, Mr. Ian Mason, and Mr. Robert Palmer from CSIRO in Canberra; Dr. Walter Boles and Ms. Jaynia Tarnawski from the Australian Museum in Sydney; and Dr. Ron Johnstone from the Western Australian Museum for their help and confidence in my abilities to spectro eggs. Of the hundreds of eggs I handled, I only cracked one, though I am reluctant to admit to which one.

I had several volunteers help me in the field, and I particularly want to acknowledge Mr. Julian Robinson for not only his spectacular wildlife photos, but also for his curiosity, zest, and friendship.

This research was made possible with funding from Birds Australia. The Canberra Ornithologists Group (COG) provided nesting records for target species within the ACT, which highlighted several white-plumed honeyeater 'hotspots'. For help with dusky woodswallows, Dr. Rachel Sims was generous enough to lend me a few of her colonies in Namadgi.

Most of all, I want to thank my family for their guidance and encouragement in my studies, travels, and life's endeavours. I am especially grateful for my partner, Seth Coluzzi, and for his remarkable support through every leg of this process—from first suggesting coming to the ANU, to holding the ladder when I was at nests, and providing endless ideas and advice; it is because of him that I am here. Thank you, Seth, for everything.

Canberra is known as Australia's bush capital, and may it remain so. It is to the longevity of the wildlife of the ACT that this work is dedicated.

Abstract:

Brood parasites lay their eggs in the nests of other species, abandoning their young entirely to the care of the host. The host pays a heavy cost for the upbringing of the foreign chick and as a result evolves defences which are specifically tuned to reduce parasitism, the most notable of which is the discrimination and rejection of foreign eggs. However, parasites respond with counter-defences such as egg mimicry, aimed at evading the host's defences. This battle between the two players segues into a coevolutionary arms race.

When cuckoos evolve highly mimetic eggs, are host's egg discrimination defences defeated? One contentious idea is that some hosts have taken a further step in the arms race, by laying eggs that look different from the parasitic eggs, thus facilitating egg discrimination. This is based on two theories. The first is that hosts maximise their chances of detecting a cuckoo egg by reducing variation within their clutches—if eggs within a clutch look similar to one another, a foreign egg would appear more distinctive. The second theory argues that increased variation between clutches in a population decreases cuckoo egg mimicry because when clutches in a population look different from each other it is harder for a parasite to mimic any particular clutch. Previous studies have found evidence both for and against these theories, but many are limited in their usefulness due to the use of subjective human assessments of clutch variation. The aim of this study is to test these ideas, through a combination of field experiments and broad scale analyses of clutch variation between populations experiencing different rates of parasitism.

An overview of cuckoo parasitism is provided in the first chapter of this thesis. Here I illustrate the coevolutionary arms race by presenting descriptions of several cuckoo 'tricks,' balanced by a few well-documented—as well as putative—host defences.

Chapter two is based on work conducted in field sites located in southeast Australia and combines data collected over two breeding seasons (2006–8). By performing clutch manipulation experiments on several pallid cuckoo *Cuculus pallidus* hosts (white-plumed honeyeaters *Lichenostomus penicillatus*, a 'major' host, and dusky woodswallows *Artamus cyanopterus*, and willie wagtails *Rhipidura leucophrys*, 'occasional' hosts) I found that the rate of foreign egg rejection was primarily influenced by the degree of difference in colour between the foreign egg and host clutch, and the amount of within-clutch variation was no different between individuals who rejected and those who accepted the foreign egg. This is the first study, to my knowledge, to investigate the extent of rejection abilities in these host species. Since these species are parasitised to varying degrees I was also able to determine that the major host (i) did not reject foreign eggs at a higher rate but was able to reject eggs of more similar appearance, (ii) had *greater* within-clutch variation (in opposition to the hypothesis), (iii) but also had *greater* variability between clutches in the population (supporting the hypothesis).

In the third chapter, this field study is supplemented by data collected from museum egg holdings. I analysed clutches of four pallid cuckoo host species, three of which are parasitised more heavily in one part of their range, but less frequently in another. By utilising this discrepancy in rate of parasitism between populations of the same species, I found that high rates of parasitism do not drive changes in overall egg colour, but do tend to decrease within-clutch variation and increase between-clutch variability, supporting theoretical predictions.

In the general discussion I synthesise findings from 21 previous studies and this study, and conclude that at present the hypothesis that parasitism should select for decreased within-clutch variation in hosts is only weakly supported, whereas there is now more conclusive support for the theory that parasitism increases variation between

clutches in a population. Finally, I make suggestions for future studies and possible directions.

Table of Contents

Chapter 1:

Introduction

> 'it lays its eggs in the nest of smaller birds after devouring these birds' eggs'
>
> 'they do not sit, nor hatch, nor bring up their young, but when the young bird is born it casts out of the nest those with whom it has so far lived'

Baffling observations of the European cuckoo *Cuculus canorus* have been recorded since classical times and include these by Aristotle (384–322 BCE) (Aristotle trans. Hett 1936, p241; Aristotle trans. Peck 1970, p251; cited in Davies 2000). He took notice of the peculiar nature of this brood parasite, a bird that never raises its own young but relies on other species to nest build, incubate, and care for its chicks (Wyllie 1981; Davies & Brooke 1988). For centuries curiosity has led naturalists to mull over the nesting habits of the European cuckoo until Edgar Chance's extensive and detailed field observations (1918–1925) not only confirmed many speculations but also revealed novel findings (Chance 1922; 1940).

Chance was the first to realise that the female cuckoo carefully monitors the nesting progress of a potential host. She waits until the host is away before secretly alighting on the side of the nest, where she removes one egg and while holding it in her bill quickly lays one of her own, directly into the nest cup (Chance 1922). The visit is brief, generally lasting no more than 10 seconds (Chance 1922; 1940; Molnár 1944; Seel 1973; Wyllie 1981). The cuckoo typically hatches before the native clutch (Chance 1922; Payne 1977) and a scant eight to ten hours after emerging from its shell - performs a miraculous feat (Molnár 1944): the hatchling, naked and blind, balances the hosts' eggs (or young chicks) on its back, moves to the edge of the nest, and one by one tosses them over the side—a performance first documented by Edward Jenner (1788). The task of evicting all the remaining host eggs falls to the cuckoo chick rather than the female cuckoo, since hosts desert single eggs but not single chicks (Davies & Brooke

1988). As the sole inhabitant of the nest, the young cuckoo demands the complete attention of the host and often quickly becomes many times larger than the fosterer (Jenner 1788). This size difference can be stunning, as many hosts weigh approximately 15 percent of the weight of an adult European cuckoo (Wyllie 1981).

AVIAN BROOD PARASITISM

The European cuckoo is the most widely studied avian obligate brood parasite, relying solely on other birds to care for and raise its young. However, this rare reproductive strategy originated independently in at least six different bird families and extends to 80 known species, which amounts to about 1% of all bird species (Wyllie 1981; Davies 2000). The honeyguides, African *Vidua* finches, and even the South American black-headed duck, *Heteronetta atricilla*, highlight the diversity found in obligate brood parasites. The family the European cuckoo belongs to, Cuculidae, is composed of around 140 species, yet only 57 are parasitic (Davies 2000). Of these, 11 known species breed in Australia (Brooker & Brooker 1989).

COEVOLUTIONARY ARMS RACE

The host of a cuckoo pays a high cost if successfully parasitised. Not only are all the native eggs and chicks turned out of the nest, but the fosterers must spend a considerable amount of energy and time rearing the foreign bird (Payne 1977). A single young cuckoo increases in weight as rapidly as a whole brood of host chicks, and remains dependent on the host parents for a significantly longer time (Wyllie 1981). To offset this heavy loss caused by parasitism, natural selection promotes the development of host defences. These defences in turn select for improved counter measures by the cuckoo resulting in coevolution of the host and parasite (Janzen 1980; Brooke & Davies 1988; Davies & Brooke 1989a; 1989b); both players become more refined in order to

out-compete the other, thus creating an evolutionary arms race between the cuckoo and host species (Payne 1977; Dawkins & Krebs 1979; Rothstein 1990).

HOST DEFENCES: AGGRESSION, DETECTION, LEARNING, AND REJECTION

Host defences fashioned by coevolution are a direct response to, or are maintained by, selection pressures specifically resulting from parasitism (Janzen 1980; Rothstein 1990). An example of a tactic a host can use to defend its nest and prevent cuckoos from laying is recognising the adult cuckoos and acting aggressively towards them (Moksnes *et al.* 1990; Duckworth 1991; Røskaft *et al.* 2002; Røskaft *et al.* 2002).

Perhaps the most effective defence a host has against brood parasitism, however, is the detection and rejection of foreign eggs (Rothstein 1975; Davies & Brooke 1989a). One cause of egg rejection by a host includes sighting a cuckoo near the nest, in which case they are more likely to reject an egg if it looks suspicious. Also, hosts will reject any egg that appears in the nest before they begin laying their own clutch (Davies & Brooke 1988).

One theory suggests that birds may use unique shell spotting patterns as a way to distinguish eggs (Swynnerton 1918; Cherry *et al.* 2007). Though Davies and Brooke (1989b) did not find evidence of more distinct spotting patterns in species with a history of interaction with cuckoos, there is supporting evidence that hosts learn to recognise their own eggs and thereby are able to distinguish foreign eggs (Rothstein 1975; Lotem *et al.* 1992). For example, African village weaverbirds *Ploceus cucullatus* were shown to eject entire clutches of foreign eggs, suggesting their discriminatory abilities are not based on 'discourse'; they do not simply use direct comparison to reject odd eggs in a clutch but discriminate based on memory of the colour and speckling of their own eggs (Victoria 1972; Jackson 1998; Lahti & Lahti 2002). Also, in a population of great reed warblers, naïve first-year breeders accepted more foreign eggs than older breeders who

had time to learn the appearance of their own eggs (Lotem *et al.* 1992; 1995). However Amundsen *et al.* (2002) failed to find any evidence for learning in the host species the bluethroat *Luscinia svecica*.

Forms of rejection in response to brood parasitism include grasping or pecking at and ejecting the egg from the nest with their beak, weaving nest materials on top of the parasitic egg (as well as possibly over their own eggs) to prevent hatching, and complete nest abandonment (Baker 1913; 1942; Rothstein 1975; Davies & Brooke 1988; Hill & Sealy 1994; Sealy 1995).

CUCKOO COUNTER-ADAPTATIONS: TIMING

Cuckoo counter-defences are those adaptations that respond to advances in host defences (Janzen 1980; Rothstein 1990). Timing is very important for successful parasitism. Hosts will reject any eggs that appear before their own clutch begins, thus cuckoos correspondingly delay their laying until the host initiates laying (Wyllie 1981; Davies & Brooke 1988). However, cuckoos that lay too long after their host begins are at a disadvantage, either because their chick would be less likely to hatch before the host chicks, and chicks are more difficult to turn out of a nest than eggs (Davies & Brooke 1988), or because hosts desert their clutch if visited at the nest by a cuckoo during incubation (Langmore *et al.* 2003).

Timing is also essential on the day the cuckoo lays in order to counter host aggression and augmented egg rejection. The female cuckoo must secretly observe the host from a nearby vantage point then visit the nest during the afternoon when the host is likely to be absent. The cuckoo spends only a few fleeting seconds laying her egg, while in comparison other birds can take up to an hour (Chance 1940; Molnár 1944; Seel 1973; Wyllie 1981; Davies & Brooke 1988).

HOST SUITABILITY AND FREQUENCY OF PARASITISM

There are several minimum requirements a host must meet to successfully rear a cuckoo. These include building a nest accessible for parasitism, and providing food the cuckoo chick can digest (invertebrates, especially insects) (Davies & Brooke 1989a; Moksnes *et al.* 1990). Since host defences evolve from exploitation by cuckoos, unsuitable hosts exhibit either reduced or a complete lack of defences against parasitism, such as showing less aggression towards a cuckoo mount than suitable hosts (Moksnes *et al.* 1990; Røskaft *et al.* 2002), and failing to reject odd eggs in their nest (Davies 2000).

Host defences also evolve proportionally with frequency and exposure to brood parasitism (Lindholm & Thomas 2000). Within species of suitable hosts, parasitised populations showed stronger discrimination and rejection abilities than populations isolated from cuckoos (Davies & Brooke 1989b). For example, Soler and Møller (1990) verified that populations of parasitised magpies *Pica pica* in long-standing sympatry with great spotted cuckoos *Clamator glandarius* demonstrated greater rejection than populations in areas of recent sympatry.

REJECTION COSTS

Due to the greatly reduced reproductive fitness caused by successful parasitism, ejecting a parasitic egg can greatly benefit the host bird if the foreign egg is easily distinguishable and can be removed without damaging the rest of the clutch. However, ejection can incur costs if the host mistakenly casts out its own egg (Molnár 1944; Davies & Brooke 1988), or damages its own eggs while trying to grasp or puncture the parasitic egg (Davies & Brooke 1988; Moksnes *et al.* 1991). These costs prevent less heavily parasitised hosts from rejecting. According to the evolutionary equilibrium hypothesis, hosts accept parasitic eggs when the costs of rejection are greater than the

benefits (Davies & Brooke 1988; Lotem *et al.* 1992). Studies by Rothstein (1975) suggest some genetic component behind egg rejection, with species being classified as either egg 'rejectors' or 'acceptors'. However, phenotypic flexibility in egg rejection is evident when different conditions shift the cost-benefit balance, allowing rejection rates to vary between populations as well as individuals (Davies & Brooke 1988; 1989b; Lotem *et al.* 1995; Davies *et al.* 1996). In other words, when the likelihood of parasitism decreases, rejection rates also decline. This plasticity in rejection behaviour is adaptive, especially given the costs of rejection and the patchiness of parasite abundance (Davies *et al.* 1996; Brooke *et al.* 1998; Lindholm & Thomas 2000).

EGG DISCRIMINATION AND EFFECT ON CUCKOO EGG MORPHOLOGY

Size and strength of the cuckoo egg

Parasitic cuckoos are typically larger than their hosts, which would potentially create a significant size discrepancy between the host egg and parasite egg. However, a comparison of 28 parasitic cuckoo species and 43 nesting cuckoo species shows that parasitic cuckoos lay smaller eggs than non-parasitic cuckoos of the same body size (Payne 1974). This was supported in a more recent analysis by Krüger and Davies (2004), which controlled for phylogenetic relatedness.

Though parasitic cuckoos lay remarkably small eggs for their body size, the eggs are still slightly larger than most of their hosts', yet the hosts often do not reject them. An experiment by Davies and Brooke (1988) showed that reed warblers *Acrocephalus scirpaceus* discriminate against very large model eggs that weigh 10 g. However, they tolerate model eggs that weigh the same amount as a European cuckoo egg (3.4 g), which are only slightly larger than their own eggs. For some host species, however, egg size is a stronger cue for egg rejection than colouration and spotting (Marchetti 2000). In species with dark, dome-shaped nests, detection of cuckoo eggs is constrained by

poor visibility. Hosts such as superb fairy-wrens *Malurus cyaneus* can still detect larger eggs using tactile cues, and this appears to be the only signal, other than mistimed laying, that is used in egg-rejection decisions (Langmore *et al.* 2003).

Besides matching host eggs more closely, there are likely other benefits to having small eggs, such as allowing for reduced incubation length (Rahn & Ar 1974), a feature that allows cuckoo chicks to hatch before it is too late to push the host eggs or small chicks from the nest (Davies & Brooke 1988). Also, large eggs are more costly for cuckoos to form as well as possibly being more difficult for small hosts to incubate (Davies & Brooke 1988).

In addition to laying relatively small eggs, parasitic cuckoos have also evolved peculiarly strong eggshells (Lack 1968; Spaw & Rohwer 1987; Brooker & Brooker 1991). This adaptation may possibly reduce damage during laying, particularly since the female cuckoo lays quickly, or may have to lay her egg through a narrow nest entrance and let it fall a short distance (Lack 1968). More likely, a recent study by Antonov *et al.* (2006) shows that a thicker and stronger shell makes puncture ejection difficult, increasing the chances of damaging the host eggs. This cost may cause some hosts to accept a suspicious egg rather than risk damage to their own eggs (Spaw & Rohwer 1987).

Egg mimicry

Mimicry is a resemblance between two unrelated species that results from coevolution (Payne 1967; Grim 2005). Baker was the first to propose that hosts eliminate foreign-looking eggs, selecting for cuckoo eggs that are the most strikingly similar to their own (Baker 1913; 1923; 1942). Since Baker's time there has been strong supporting evidence for this (Brooke & Davies 1988; Davies & Brooke 1988; Higuchi 1989; Moksnes *et al.* 1990; Lotem *et al.* 1995; Lahti & Lahti 2002). As the

degree of contrast between host and parasitic eggs decreases, fewer foreign eggs should be rejected (Baker 1942; Honza *et al.* 2004).

Evolution of polymorphic cuckoo eggs and gentes

Some cuckoos are specialists, parasitising only one host species and evolving eggs that closely resemble that single host's eggs. However, others, such as the European cuckoo, are considered generalists and can parasitise multiple sympatric species. To reduce host rejection, it appears that many generalists lay polymorphic eggs: they evolve host-specific races called gentes ('gens' for singular) within their own species. The females in each gens lay eggs which match those of their particular host species (Jourdain 1925; Chance 1940; Baker 1942; Wyllie 1981; Brooke & Davies 1988; Davies & Brooke 1988; Gibbs *et al.* 2000).

HOST INTRACLUTCH AND INTERCLUTCH VARIATION

Mimicry of host eggs by cuckoos can be remarkably accurate (eg. Starling *et al.* 2006), provoking the question of whether there are any further steps that hosts can take in the arms race against cuckoos. One proposed, but contentious, adaptation to facilitate the discrimination of parasitic eggs is the reduction of variation in egg appearance within host clutches (intraclutch variation) and the increase in clutch variation among hosts in a population (interclutch variation) (Victoria 1972; Davies & Brooke 1989b; Øien *et al.* 1995). This theory assumes that (i) a foreign-looking egg is easier to pick out of a clutch of uniform looking eggs (Davies & Brooke 1989b; Stokke *et al.* 1999; Kilner 2006) and (ii) high variation among clutches makes it harder for the cuckoo to mimic the eggs of a particular individual (Swynnerton 1918; Davies & Brooke 1989b; Honza *et al.* 2004; Kilner 2006).

Many analyses have considered alternative roles of intraclutch and interclutch variation, including their significance in host female age and learning (Lotem *et al.* 1995) and the way they evolve with conspecific brood parasitism (Victoria 1972; Freeman 1988; Møller & Petrie 1991; Jackson 1998). With this in mind, the true utility of differences within and between clutches in egg discrimination remains unknown.

Over the past 35 years multiple studies have specifically investigated the way cuckoo parasitism has affected differences in eggshell appearance within and between host clutches. These studies can be separated into those that compare groups of species (Table 1.1) and those that focus on populations or individuals within a species (Table 1.2). Many investigations relied on subjective human assessments of colour, so the results need to be interpreted with caution. However, within the remaining studies, much ambiguity remains over the effects of parasitism on within-clutch variation and between-individual variability.

HOW CAN WE QUANTIFY EGG MIMICRY?

Visual cues are important to hosts for discriminating their own eggs from foreign eggs; however, avian vision is very different from that of humans (Goldsmith 1990). It is important to avoid human perception biases when dealing with non-human species (Bennett *et al.* 1994; Cuthill *et al.* 1999; Cuthill *et al.* 2000), since signals are only interpreted by the individual receiving them (Endler 1978; 1990). Thus, a further complicating factor in detecting mimicry and degree of clutch variation is that it is necessary to 'see' the world the same way that the organism receiving the signal sees it (Cuthill & Bennett 1993; Dittrich *et al.* 1993).

Unlike most vertebrates, humans are normally blind to ultraviolet light (Bennett & Cuthill 1994; Bennett *et al.* 1994). We are trichromatic, having only three cone types in our retina, which allow us greatest sensitivity to red, green, and blue light (reviewed

in Dalton 2004). Diurnal birds, however, are endowed with at least four distinct types of single cone as well as a double cone (reviewed in Hart & Hunt 2007), which enable them to have one of the richest capacities for colour vision within the vertebrates (Goldsmith 1990; Kelber *et al.* 2003; Hart & Hunt 2007). In many bird species these four visual pigments absorb maximally at ca. 565, 480, 430–450 and 360–380 nm (Chen & Goldsmith 1986; Bowmaker *et al.* 1997). While the 'human-visible' part of the electromagnetic spectrum ranges from ca. 400–700 nm, bird vision includes ultraviolet light (300–400 nm) and thus spans ca. 300–700 nm (Bennett & Cuthill 1994). It is this 360–380 nm cone that provides birds their sensitivity to ultraviolet light, as was first demonstrated in the pigeon *Columba livia* (Wright), and in the hummingbird *Colibri serrirostris* (Huth & Burkhardt), both in 1972. This UV vision is so strong that in the red-billed leiothrix *Leiothrix lutea* the 380 nm sensitivity is more than five times greater than the maximum sensitivity of the bird in the human-visible range (Burkhardt & Maier 1989; Maier 1992).

Hypotheses about the function of UV vision in birds include use in orientation, foraging, and sexual selection (reviewed in Bennett & Cuthill 1994), though recent studies have revealed many more complexities and applications of avian vision (Cherry *et al.* 2007), including detection of chick gape colours (Hunt *et al.* 2003) and egg recognition (Cherry & Bennett 2001; Cherry *et al.* 2007).

Spectrophotometry

Since the growing awareness of the differences between human and avian colour vision of the past decade, fundamental changes have revolutionised the way colour is measured, making it independent of human colour perception. In order to assess egg appearance objectively, it is prudent to include the use of reflectance spectrophotometers sensitive to ultraviolet light so that they encompass the entire bird-

visible waveband (300–700 nm). Several studies have hence incorporated the use of bird-visible reflectance spectrophotometry to reveal subtle spectral properties of eggs that were previously undetected by humans (Cherry & Bennett 2001; Soler *et al.* 2003; Avilés & Møller 2004; Starling *et al.* 2006).

Museum experiments have also used reflectance spectrophotometry to detect UV egg mimicry invisible to humans, which gives clues to why some hosts accept seemingly non-mimetic eggs (Cherry & Bennett 2001). In contrast, Aviles *et al.* (2006) experimentally manipulated the eggs of great spotted cuckoos *Clamator glandarius* using a UV light blocker and used them to parasitise a population of magpies, *Pica pica*. They found no significant differences between rejection rate of cuckoo eggs with and without reduced reflectance in the UV region, which suggest UV cues alone do not effectively signal parasitism to magpies.

A disadvantage of reflectance spectrophotometry is that it does not fully describe egg maculation. The spectrophotometer averages the reflectance of all colours that lie in the area of the probe (1 mm^2) such that hue from speckling is averaged with ground colour in the spectrum reading. Spot size, density, distribution and colour are not separately determined and cannot be analysed independently from ground colour. One method used to incorporate patterns of maculation is to include human visual assessments to rank and score egg maculation (Lahti 2005; Avilés *et al.* 2006; Cherry *et al.* 2007). Nevertheless, like all other human-evaluated techniques, this methodology still fails to objectively quantify spotting.

Objectively measuring clutch variation

Recent analyses of clutch variation have included the use of spectrophotometers to detect slight differences at human-visible wavelengths (Avilés *et al.* 2004) as well as ultraviolet wavelengths (Avilés & Møller 2003) to which a human assessor would be

blind. This technique reveals important aspects of clutch variation that would otherwise remain cryptic (Cherry *et al.* 2007).

It is essential to note that all of the interspecific studies on how clutch variation affects rejection rate (Table 1.1) used human based measurements of egg appearance. Only seven of the 16 intraspecific studies (Table 1.2) used some sort of objective methodology to quantify egg appearance, and five of these were via reflectance spectrophotometry, which includes the ultraviolet spectrum. The other two experiments (Soler *et al.* 2000; Moskát *et al.* 2002) used a computer image analysis program to measure the colour value of each egg. In total, five of these seven studies support the prediction that intraclutch variation in at least one chroma decreases as a result of cuckoo parasitism, and the other two found that intraclutch variation increases(Avilés *et al.* 2004; Cherry *et al.* 2007). Only two of the seven objective studies include aspects of interclutch variation, but both support the hypothesis that interclutch variation increases in response to parasitism. These seven objective studies are more meaningful and reliable than those based purely on human vision.

EVOLUTION OF EGG POLYMORPHISM IN HOSTS

It is well documented that several genera of cuckoo lay polymorphic, host-specific egg types, such as the European cuckoo, the Asiatic large hawk-cuckoo *Cuculus sparverioides*, the red-chested cuckoo *Cuculus solitarius* (Jourdain 1925; Chance 1940; Baker 1942; Wyllie 1981; Brooke & Davies 1988; Davies & Brooke 1988; Cherry *et al.* 1998; Kuiper & Cherry 2002), and the diederik cuckoo *Chrysococcyx caprius* (Lawes & Kirkman 1996). Several heavily parasitised host species have also been noted to have high interclutch variation, such as the African village weaverbird *Ploceus cucullatus* (Victoria 1972). This within-species variation arises when cuckoo mimicry (or conspecific parasitism) is so advanced that the only

way for hosts to recognise their own eggs is to lay eggs that look unlike the rest of the host population (which the parasite matches). Coevolutionary pressure will eventually split the host populations into various discrete egg morphs (Swynnerton 1918; Davies & Brooke 1989b; Collias 1993; Soler & Møller 1996; Takasu 2003; Kilner 2006). While there is relatively little field data surveying the extent of egg polymorphisms, Takasu (2003) developed a model predicting this convergence of discrete egg polymorphisms in both parasite and host resulting from coevolution.

Though egg appearance may be constrained in some hosts by selection pressures such as the need for crypsis and protection from solar radiation (Møller & Petrie 1991; Underwood & Sealy 2002), diversity in egg types may also increase for similar reasons. Predation and the utilisation of an array of nest sites are two primary factors that contribute to egg variation (Kilner 2006). Brood parasitism secondarily leads to discrete egg polymorphisms by possibly selecting for more uniform clutches (Kilner 2006), consequently exaggerating existing interclutch variation (Soler & Møller 1996; Stokke *et al.* 1999). Unfortunately very few studies have quantitatively addressed the formation of egg polymorphisms resulting from brood parasitism (Takasu 2003).

Geographic variations and polymorphisms in hosts and cuckoos

Natural deviations in egg appearance allow bird populations to adapt to local habitat conditions. As briefly mentioned above, environmental conditions and habitat structure influence aspects of how eggs look, such as in adaptations for egg thermoregulation and crypsis from predators. These evolutionary constraints gradually filter and shape genetically-determined variables that affect eggshell colour and patterning (Kilner 2006), so that eggs become suited to their own geographic region. Because of this, it may be possible that interclutch variation can bring about geographic

variations in host populations and also lead to the creation of stable host egg polymorphisms.

In those cuckoo-host systems in which the hosts have evolved high rejection rate and high interclutch variation (such as the European cuckoo and its European hosts), a cuckoo gens may theoretically split off into additional gentes, each one specialising in and resembling one host morph (Stokke *et al.* 2005).

PROJECT BACKGROUND

I will investigate several aspects of the coevolution between the pallid cuckoo *Cuculus pallidus* and its host species. An Australian native, the pallid cuckoo occurs throughout the continent and inhabits all types of open country where there are trees. Weighing 80–85 g, it is a medium-large cuckoo about 31 cm long, and is a little studied obligate brood parasite. As a generalist, it parasitises a wide range of hosts, and is recorded to have exploited over 100 different species of passerines—though all major hosts belong to the Meliphagidae (Brooker & Brooker 1989).

Unlike the European cuckoo, the pallid cuckoo was believed to lay only one egg type: a pale pink egg sprinkled with sparse dots of a darker hue. Recent evidence, however, suggests they have 'cryptic gentes' (Starling *et al.* 2006). The gentes mimic minor variations between the four closely related primary host species tested, which were all melaphagid honeyeaters: the white-plumed honeyeater *Lichenostomus penicillatus,* the yellow-faced honeyeater *Lichenostomus chrysops,* the yellow-tufted honeyeater *Lichenostomus melanops* and the black-headed honeyeater *Melithreptus affinis*. The differences between the gentes are so subtle they are only detectable using reflectance spectrometry. Since egg mimicry by cuckoos evolves as a response to egg rejection by hosts (Davies 2000), this extreme accuracy of egg mimicry by the pallid

cuckoo suggests many of the hosts are discriminating egg rejectors (Starling *et al.* 2006).

OBJECTIVES

Field experiments

Experiments conducted in the field involve manipulating clutches of several host species to compare foreign egg rejection of a 'major' pallid cuckoo host, the white-plumed honeyeater *Lichenostomus penicillatus*, with that of two 'occasional' hosts, dusky woodswallows *Artamus cyanopterus* and willie wagtails *Rhipidura leucophrys*. I measure eggshell colour objectively by using a reflectance spectrophotometer.

1) The first part of my project will be to determine if pallid cuckoo hosts reject foreign eggs, and if so, I will examine several factors believed to increase rate of rejection. These factors include high rate of parasitism suffered by the host, high contrast between foreign egg and host clutch, and low within-clutch variation.

2) I next examine if rate of parasitism is influential in reducing variation within host clutches and increasing variability between clutches in a host species.

Museum egg collections

These field experiments will be complemented by quantitative analyses of museum egg collections, and investigate the impact of parasitism on changes in eggshell colouration. Analyses will include four pallid cuckoo hosts (singing honeyeaters *Lichenostomus virescens*, red wattlebirds *Anthochaera carunculata*, yellow-throated miners *Manorina flavigula*, and yellow-tufted honeyeaters *Lichenostomus melanops*), and compare two geographically separated populations of each. The populations are parasitised to varying degrees—for example, the red wattlebird *Anthochaera carunculata* is a primary host in south-western Australia but is uncommonly parasitised

in south-eastern Australia (Brooker & Brooker 1989). This makes it possible to compare the effects of parasitism on changes in egg colouration. As with the field experiments, egg colour is measured using reflectance spectrophotometry.

3) I will first determine if parasitism influences changes in the general egg colour of a host population. In response to mimicry by cuckoos, we may discover hosts avoid parasitism by changing their own egg colour, so it may be possible that populations with the greatest amount of difference in their parasitism rate also have the greatest amount of difference between their mean egg colour.

4) To test whether parasitism by pallid cuckoos has led to the evolution of low intraclutch variation in hosts, I will compare the amount of within-clutch variation in occasionally parasitised populations with populations of the same host species that suffer from more frequent parasitism.

5) I will then test whether parasitism by pallid cuckoos has driven the evolution of high interclutch variation or even host egg polymorphisms. Thus I predict that the more heavily parasitised population would show greater between-clutch variation or egg polymorphisms than their counterparts across the continent.

Table 1.1. Interspecific studies: amount of within-clutch variation and between-clutch variation of cuckoo host species compared to non-host species

Study	Species	Mode of assessment	Within-clutch variation of cuckoo hosts	Between-clutch variation of cuckoo hosts	Notes
(Davies & Brooke 1989b)	7 suitable versus 5 unsuitable	Eye	No significant relationship	No significant relationship	
(Øien *et al.* 1995)	47 suitable versus 28 unsuitable	Eye	No significant relationship	Increased	
(Soler & Møller 1996)	47 suitable versus 28 unsuitable	Eye	Decreased	Increased	Based on Oien 1995 but controlling for phylogenetic relatedness
(Stokke *et al.* 2002)	99 suitable European passerines versus 122 North American passerines	Eye	No significant relationship	Increased	Comparative study between European passerines parasitised by mimetic cuckoos and North American passerines parasitised by non-mimetic cowbirds

Table 1.2. Intraspecific studies. Experiment type 1: Changes in within-clutch and between-clutch variation of acceptor versus rejector individuals within a population. Experiment type 2: Changes in within-clutch and between-clutch variation of parasitised versus non-parasitised populations (sympatric versus allopatric). Experiment type 3: Experimentally increased within-clutch variation (by swapping conspecific eggs) and compare rejection rate of artificial cuckoo eggs between manipulated versus un-manipulated (control) conspecific nests

Study	Species	Expt type; Type of egg used to test rejection	Mode of assess-ment	Within-clutch variation of rejectors	Between-clutch variation of rejectors	Notes
(Victoria 1972)	African village weaverbird (*Ploceus c. cucullatus*)	1 Conspecific eggs, varying degrees of mimicry	Eye	N/A	N/A	Population has naturally high between-clutch and low within-clutch variation
(Davies & Brooke 1989b)	Meadow pipit (*Anthus pratensis*) & pied/white wagtail (*Motacilla alba*)	2	Eye	No significant relationship	No significant relationship	
(Stokke *et al.* 1999)	Reed warbler (*Acrocephalus scirpaceus*)	1 Artificial non-mimetic cuckoo egg	Eye	Decreased	N/A	Moderate rejection of non-mimetic eggs
(Soler *et al.* 2000)	Black-billed magpie (*Pica pica*)	1 Artificial non-mimetic egg	Computer-image analysis program	Decreased	N/A	Population has naturally high between-clutch and low within-clutch variation
(Moskát *et al.* 2002)	Great reed warbler (*Acrocephalus arundinaceus*)	2	Computer-image analysis program	Decreased	Increased	Sympatric population is heavily parasitised
(Avilés & Møller 2003)	Meadow pipit (*Anthus Pratensis*)	2	Reflectance spectro-photometer	Decreased	N/A	Moderate rejection of non-mimetic eggs; only UV chroma is significantly less variable in rejector clutches
(Karcza *et al.* 2003)	Great reed warbler (*Acrocephalus arundinaceus*)	3 Artificial moderately mimetic cuckoo egg	Eye	No significant relationship	N/A	Species has naturally high between-clutch variation; heavily parasitised population

(Procházka & Honza 2003)	Common whitethroat (*Sylvia communisw*)	1 Mimetic conspecific egg	Eye	No significant relationship	N/A	Species has naturally low within-clutch variation and high between-clutch variation; ejects all non-mimetic eggs
(Avilés *et al.* 2004)	Common magpie (*Pica pica*)	1 Artificial cuckoo egg that appears mimetic to humans (spectrum is non-mimetic)	Reflectance spectro-photometer	Increased	N/A	Only green chroma is significantly more variable in rejector clutches
(Honza *et al.* 2004)	Blackcap (*Sylvia atricapilla*)	1 Moderately mimetic conspecific eggs were used in the comparison	Eye	No significant relationship	N/A	Species has naturally low within-clutch variation and high between-clutch variation; ejects most non-mimetic eggs
(Lovászi & Moskát 2004)	Red-backed shrike (*Lanius collurio*)	1 Conspecific eggs, varying degrees of mimicry	Eye	No significant relationship	N/A	Species has naturally high between-clutch variation
(Procházka & Honza 2004)	Yellowhammer (*Emberiza citronella*)	1 Mimetic conspecific egg	Eye	No significant relationship	N/A	Ejects most non-mimetic eggs
(Stokke *et al.* 2004)	Chaffinch (*Fringilla coelebs*)	1 Moderately mimetic conspecific eggs were used in the comparison	Eye	No significant relationship	N/A	Species has naturally low within-clutch variation and high between-clutch variation; ejects most non-mimetic eggs
(Lahti 2005)	African village weaverbird (*Ploceus cucullatus*)	2	Reflectance spectro-photometer Eye (for maculation only)	Decreased	Increased	Species has naturally low within-clutch variation and high between-clutch variation
(Antonov *et al.* 2006)	Marsh warbler (*Acrocephalus palustris*)	1 Five egg types of varying mimicry	Eye	No significant relationship	N/A	Heavily parasitised; ejects most non-mimetic eggs

(Cherry *et al.* 2007)	Great reed warbler (*Acrocephalus arundinaceus*)	1 Naturally parasitising mimetic cuckoo eggs	Reflectance spectro-photometer Eye (includes maculation)	Increased	N/A	UV chroma is more variable in rejector clutches; Heavily parasitised population
(Polačiková *et al.* 2007)	Blackcap (*Sylvia atricapilla*)	1 Moderately mimetic conspecific eggs	Reflectance spectro-photometer Eye (includes maculation)	No significant relationship when assessed with eye Decreased when assessed with spectro-photometer	N/A	Only blue chroma is significantly lower in rejector clutches; naturally low within-clutch variation and high between-clutch variation; not commonly parasitised but are good rejectors

Chapter 2:

Egg discrimination and clutch variation in three pallid cuckoo hosts that experience different rates of parasitism

Introduction:

In many cuckoo species, the nestling cuckoo evicts the host eggs and chicks from the nest (Davies 2000). Thus victims of successful parasitism lose their entire brood as well as invest substantial time and energy rearing the parasitic chick. This reduced fitness provides a selection pressure for hosts to develop foreign egg recognition and rejection abilities (Davies 2000). In response to this discrimination by the host, the parasite evolves more highly mimetic eggs, which decreases the amount of contrast in colour and patterning when compared to the host eggs (Baker 1913; Brooke & Davies 1988). Reduced contrast makes recognition of the foreign egg more difficult for the host and increases the likelihood of recognition errors (Brooke & Davies 1988; Davies & Brooke 1988; Lotem *et al.* 1995). Davies et al. (1996) demonstrated that the probability of rejection of mimetic foreign eggs decreases with increasing likelihood that the host will make recognition errors, and increases with parasitism rate.

In theory, highly mimetic cuckoo eggs could, in turn, select for changes to host clutches. Specifically, hosts could retaliate against cuckoo egg mimicry by reducing the amount of variation between the eggs in their clutch; this theory assumes that a foreign egg is more noticeable in a clutch of uniform-looking eggs (Davies & Brooke 1989b; Stokke *et al.* 1999; Kilner 2006).

As variation within individual clutches decreases, parasitism is also argued to select for increased variation between clutches in a population. If hosts lay eggs that look different from other clutches in the population, it is it harder for cuckoos to mimic the eggs of any particular individual (Swynnerton 1918; Davies & Brooke 1989b; Honza *et al.* 2004; Kilner 2006). The host clutches will theoretically diverge from the 'mean' eggshell appearance, because this is what the cuckoo egg is selected to mimic (Takasu 2003).

Although numerous studies have found evidence both for and against these two hypotheses (see Chapter 1: Table 1.1, Table 1.2), only a few recent studies have incorporated the use of reflectance spectrophotometry for objectively measuring clutch variation. Of these studies, three have supported the hypothesis that parasitism reduces within-clutch variation (Avilés & Møller 2003; Lahti 2005; Polačiková *et al.* 2007), and two resulted in the opposing view, that females who rejected foreign eggs actually had higher within-clutch variation than acceptors (Avilés *et al.* 2004; Cherry *et al.* 2007). Only one study which used reflectance spectrophotometry considered the effects of parasitism on clutch variation between females in a population (Lahti 2005), and it supported the hypothesis that parasitism increases between-clutch variation (Table 1.2).

In this study, I test the egg discrimination capabilities of several hosts of the pallid cuckoo. First, I aim to determine if these host species exhibit basic egg discrimination and rejection abilities. For those that do, I will examine several potential factors believed to influence a host's reaction when faced with a possible foreign egg. These factors include the rate of parasitism suffered by the host, the amount of contrast between the foreign egg and host clutch, and the amount of within-clutch variation of the host clutches. Finally, I will determine if parasitism rate indeed influences the putative adaptation of reduced within-clutch variation and increased between-individual variability.

Methods:

To test these aims, I performed egg-swap experiments on wild populations. These experiments involved transferring eggs between nests to simulate parasitism by a cuckoo. To test whether hosts display any egg rejection abilities, and whether the degree of mimicry affects the probability of rejection, I presented individuals of each

host species with eggs of varying levels of contrast, including conspecific and heterospecific eggs. To determine whether frequency of parasitism influences a host's ability to discriminate foreign eggs, I performed these experiments on one 'major' host and two 'occasional' hosts. Eggshell colour was measured objectively using reflectance spectrophotometry.

STUDY SPECIES SELECTION

Pallid cuckoos regularly parasitise 32 passerine species (Brooker & Brooker 1989), and of these hosts, I selected species to study in the field based on the frequency of parasitism (Brooker & Brooker 1989). I aimed to compare a 'major' host with several less frequently parasitised 'occasional' hosts to determine whether rate of parasitism affects foreign egg rejection abilities.

To identify major and occasional pallid cuckoo hosts, I used Brooker and Brooker's (1989) classification of cuckoo hosts, which is the product of a major survey of the hosts of Australian cuckoos using 5244 records of parasitism from published sources, private and museum egg collections, the RAOU (Royal Australasian Ornithologists Union) nest record scheme, the Australian Bird Banding Scheme and unpublished records. They classified biological hosts of cuckoos as a species with multiple (> 4), independent (> 1 observer, > 1 location and > 1 year) records of parasitism. Records of parasitism were allocated to blocks of 1° latitude by 1° longitude (1 block = 10 000km^2) within 12 biogeographical regions based on the boundaries of the RAOU Atlas of Australian Birds (Blakers *et al.* 1984) used to describe the distribution of Australian birds. Rankings of biological hosts included those species with more than ten records of parasitism per region and were based on rate (total number of recorded parasitisms / total number of 1° blocks with recorded parasitisms) and extent (number of 1° blocks that have records of parasitism) of observation. Those

species with a high combined ranking were designated as major biological hosts in that region.

The most commonly parasitised hosts in south-eastern Australia are *Lichenostomus* honeyeaters (Brooker & Brooker 1989). Several *Lichenostomus* honeyeaters breed in the ACT (Australian Capital Territory)—where all sites for this study are located—but the white-plumed honeyeater, *L. penicillatus*, is the most common within Canberra, ACT (Taylor 1992). White-plumed honeyeaters were also observed rearing pallid cuckoo nestlings and fledglings in Canberra on several occasions prior to this study (Langmore pers. comm.). Therefore, I elected the white-plumed honeyeater as the major host in this study.

I selected two occasional hosts, dusky woodswallows *Artamus cyanopterus* and willie wagtails *Rhipidura leucophrys*, on the following criteria. First, they are classified as biological hosts of pallid cuckoos, but not as major hosts in south-eastern Australia by Brooker and Brooker (1989). Second, they breed commonly in the ACT (Taylor 1992). Third, I took into account egg size, since in this experiment I was interested in testing egg discrimination based on egg colour, but not size. Eggs that are much larger than the foster bird's biological size range are often rejected (Davies & Brooke 1988). Both species have eggs that are not much larger than that of the major host (Table 2.1).

The egg discrimination abilities of white-plumed honeyeaters were also tested using fuscous honeyeater *Lichenostomus fuscus* eggs. Fuscous honeyeaters and white-plumed honeyeaters lay eggs that are within the same size range (Table 2.1). No experiments were performed on fuscous honeyeaters.

The relative rate of parasitism was estimated for each host species within the biogeographical grouping of South East Australia and the Murray-Darling (Table 2.2--Blakers *et al.* 1984; Brooker & Brooker 1989). These two regions were grouped by Brooker and Brooker (1989) and include the sites where the experiments were

conducted. Rate of parasitism was calculated by dividing the number of recorded pallid cuckoo parasitisms for each species by the total number of pallid cuckoo parasitisms for all species within this region.

MEASUREMENTS

Spectral reflectance measurements of both transferred eggs and experimentally parasitised clutches were taken via an Ocean Optics USB2000 spectrophotometer and PX-2 pulsed xenon light source using OOIBASE software (Ocean Optics). Reflectance was measured using a narrow-ended UV-VIS unidirectional reflectance probe with a bevelled edge, held at a constant 45° angle to the surface of the egg, and illuminated areas approximately 1.5mm in diameter. Measurements were relative to a standard white reference (WS-1 Diffuse Reflectance Standard) and to complete darkness. The spectrophotometer was re-standardised after each clutch. Integration time was set to 15ms and reflectance was taken at 2nm intervals over the range of bird-visible wavelengths (300-700nm).

Points of Measurement

Four readings were taken in two regions on each egg, the 'Side' and the 'Ring', for a total of eight measurements (Fig. 2.1). The regions were selected based on their distinct visual characteristics. 'Side' corresponds to the background colour, or the colour of the egg that makes up the majority of the egg appearance (Fig.2.1 a, b, c). Measurements were taken in the middle of the egg, somewhat above yet avoiding the region of the 'Ring'. 'Ring' refers to the concentration of dense speckling (Fig. 2.1 a) or region of darker colouration that usually appears near the base of many of the maculated eggs (Fig. 2.1 b). In those few eggs which were nearly spotless and

immaculate, the Ring was measured around the egg approximately one-third the length from the rounded base (Fig. 2.1 c).

STUDY SITES

My study sites included three locations within the ACT: Campbell Park (35°16'S, 149°10'E), Gungahlin Hill Nature Reserve (35°12'S, 149°06'E), and Lake Ginninderra (35°13'S, 149°04'E) , as well as two points in Namadgi National Park, both of which are on the Old Boboyan Rd, en route to Yankee Hat (35°44'S, 148°59'E; 35°45'S, 148°58'E). All sites comprised native eucalypt woodland except for Lake Ginninderra, which was a landscaped lakeshore. Willie wagtails and dusky woodswallows occurred at all sites, whereas fuscous honeyeaters nested in Namadgi National Park only, and white-plumed honeyeaters were absent from Namadgi. Pallid cuckoos were regularly sighted or heard calling at Campbell Park and Gungahlin Hill (Table 2.3).

CLUTCH MANIPULATION EXPERIMENTS

Experimental design

To test whether hosts display any egg rejection abilities, and to determine if the degree of contrast effects rejection rate, I presented individuals of each host species with either an egg of low contrast (egg from a different female of the same species), intermediate contrast (egg from the nest of a different species with similar eggshell colour and pattern), or high contrast (egg from a different species with a different colour and pattern).

In order to objectively classify eggs into their degree of contrast ('high', 'intermediate', or 'low' contrast), I calculated a numerical 'distance' between the experimental eggs and the host eggs. The mean reflectance spectra (Ring and Side, n =

8) of all eggs measured in the field were separated into five major chroma: UV 300–400nm; blue 400–475nm; green 475–550nm; yellow 550–625nm; red 625–700nm. The chromatic means for each egg were calculated separately for Ring and Side measurements and entered into discriminant function analyses (DFA) using JMP 6.0. DFA gives a quick reference of where the eggs of each species lie in relation to each other chromatically, and provides an objective way of measuring the multivariate 'distance' between the species (Fig. 2.2).

According to the distances between species provided by DFA (Fig 2.2), low contrast egg experiments included swapping eggs between females of the same species, eggs of intermediate contrast involved swaps between dusky woodswallow and willie wagtail eggs, and between white-plumed honeyeater and fuscous honeyeater eggs. High contrast egg comparisons comprised swaps between dusky woodswallow or willie wagtail eggs and white-plumed honeyeater eggs.

Egg-recognition experiments

Clutch manipulation experiments were conducted over two breeding seasons, between 2006 and 2008 (September through January 2007 and August through January 2008) (Table 2.3).

Nests were found by either watching for nesting behaviour and following the bird back to its nest or by simply scanning potential trees and bushes. Ideally, the nests were discovered during the building stage or during the egg laying stage. Many nests, however, were found during the incubation period. The likelihood of egg rejection appears to decline during the incubation period in some species (eg. Moksnes *et al.* 1993; Welbergen *et al.* 2001), but not others (Moksnes *et al.* 1990; Jackson 1998). Therefore the timing of parasitism was documented to test whether this affected the rejection response in these species. The age of recently laid eggs can be estimated if

they still appear slightly translucent, since it takes about four days for the shell to acquire its final opaque colour. Sometimes, however, the laying date had to be estimated after the clutch hatched by extrapolating from the average incubation length for that species (Table 2.4).

When a nest of a target species was located I used it to perform one of the three experiment types previously described. Some foreign eggs were taken from nests that were abandoned. I followed the egg-rejection experiments described in Langmore *et al.* (2005). I warmed the foreign egg (if cold) and placed it in the experimental nest. Unless an egg from that nest was used in another experiment, no additional egg was removed from the nest, since hosts do not discriminate against having an 'extra' egg (Davies & Brooke 1988; Jackson 1998). Given that hosts tend to abandon nests that have been greatly reduced in egg number (Hill & Sealy 1994), I did not remove an egg without replacement from nests containing only two eggs (which is common in honeyeaters) (Table 2.4). I left the foreign egg in the nest for five full days and if on the sixth day it was still being incubated I returned it to its original nest, if the original nest was still active. If the original nest was no longer active I placed the egg in a surrogate nest of the same species that contained eggs of roughly the same age. If no nests were available I would save the egg for the following experiment. If any of the eggs hatched, or if the nest was preyed upon before the sixth day, the experiment was excluded from the dataset.

Only one experiment was performed during each nesting attempt and no one type of experiment was performed more than once on any individual host, regardless of year. This is to eliminate any chance of imprinting on the foreign egg type and artificially reducing rejection rate in subsequent experiments (Lotem *et al.* 1992), or of alerting the host to parasite susceptibility and artificially increasing rejection rate (Hauber *et al.* 2006). Willie wagtails maintain fairly distinctive territories (Higgins *et*

al. 2006), so repeating experiments was easily avoided. Individual identity of dusky woodswallows and white-plumed honeyeaters was more difficult to determine because they nest in loose colonies (Higgins *et al.* 2001; Higgins *et al.* 2006), though pairs seemed to prefer particular sections of the colony. This was an inherent difficulty since no birds were colour-banded, save for a colony of dusky woodswallows in Namadgi National Park. I could be confident that no pair was used twice for experiments performed on this colony (n = 2 pairs). For other pairs of dusky woodswallows and white-plumed honeyeaters, pseudo-replication was avoided as far as possible by identifying pairs on the basis of timing and location of nesting. For example, if a nest was preyed upon and a pair was found building a new nest nearby, they were assumed to be the same pair.

Eggs were marked at the flattened base with a non-toxic marker to distinguish the foreign egg, and also to identify the eggs when taking spectrophotometer readings. When a clutch no longer appeared translucent (> 4 days incubation) I took reflectance spectrophotometer readings of each egg.

On the sixth day the experiment outcome was scored as either 'accepting' or 'rejecting' the foreign egg. An egg was considered accepted if it was warm and/or being incubated by the host. It was classified as rejected if the clutch was either abandoned (cold with no apparent activity) or if the foreign egg was missing or damaged.

Results of this study include outcomes of low contrast willie wagtail nest manipulation experiments conducted by Starling (2005) (n = 8), which followed the same methods.

Experiments were conducted with the approval of the ANU Animal Experimentation Ethics Committee, protocol no. F.BTZ.02.06.

STATISTICAL ANALYSIS

The results of the nest manipulation experiments were placed into two datasets, which differed in how conservatively the outcomes of the experiments were scored. While one dataset included all experiments, the more conservative dataset excluded experiments with an ambiguous outcome. The most common of these questionable outcomes was finding the nest inactive with some or all of the eggs missing. Three possible processes could lead to this outcome: (i) nest predation, (ii) abandonment of the nest by the host followed by nest predation, or (iii) egg rejection that caused accidental damage to other eggs in the brood, resulting in abandonment by the host. Exclusion of these outcomes could result in an underestimation of rejection rate, whereas including them could produce an overestimation of rejection rates. Because of this I analysed both the conservative dataset and the dataset which included the ambiguous experiments.

Principal components analysis

All reflectance spectra (Ring and Side, $n = 8$) for each egg measured in the field (eggs measured $n = 124$, total spectra $n = 992$) were entered into a single principal components analysis (PCA), and the coefficients were plotted against wavelength to depict what variation in colour is explained by each principal component. PCA, like DFA, is a method to evaluate colour that is represented in reflectance spectra, and it is useful because it summarises a large amount of data by reducing multi-dimensional data sets to fewer dimensions for analysis. It extracts patterns (factors) that explain the most variation in the data. Following the methods of Cherry *et al.* (2007) and Avilés *et al.* (2006), the first three principal components (PC1, PC2, PC3) were calculated for each spectra for each egg, and the mean score of PC1, PC2, and PC3 was determined for each egg. GenStat10 was used for all principal components analyses.

To calculate degree of egg colour matching between the host clutch and foreign egg, the difference between the mean foreign egg and host score for each experiment was calculated separately for PC1, PC2, and PC3. The standard deviations of the mean values of all the eggs in a clutch were also calculated and used as measures of intraclutch variation in eggshell colouration for each of the first three principal components. Only clutches with three eggs were considered for this analysis, because number of eggs in the clutch could also influence the magnitude of the standard deviations.

What influences rejection rate?

To determine how different factors predict likelihood of foreign egg rejection, data were analysed with logistic regression using JMP 6.0. The host's response to the experimental egg (reject/accept) was the dependent variable, and independent variables included day of 'parasitism' (number of days from when the first host egg was laid to insertion of foreign egg), the rate of parasitism experienced by the host species ('common' for white-plumed honeyeaters and 'occasional' for dusky woodswallows and willie wagtails), foreign egg matching score (for PC1, PC2, and PC3), and amount of within-clutch variation (also for PC1, PC2, PC3). Each of the first three principal components were entered in a separate model (for example, PC1 for egg matching with PC1 for within-clutch variation), along with rate of parasitism.

The effect of parasitism rate on clutch variation

The relative amount of within-clutch and between-clutch variation for the three species was assigned by means of a series of pair-wise comparisons. Using GenStat10 the three species were analysed in three comparisons involving two species each: dusky woodswallows with white-plumed honeyeaters, willie wagtails with white-plumed

honeyeaters, dusky woodswallows with willie wagtails. For each comparison, the mean reflectance spectra for each egg from the two species was entered into two separate principal components analyses (one for Ring, the other for Side) and the first three principal components were extracted for both points of measure. These six PCs were entered separately into general linear models. To account for having multiple eggs in a clutch, each clutch was given a separate nest identifier, and each egg was identified separately within each clutch. Also included were the species of the eggs. The two species were separated using 'dummy variables' so that the within-species variation could be distinguished in the same analysis. The accumulated analysis of variance was determined and this yielded the amount of variance, represented by the mean square values for the two species, associated with within-clutch and between-clutch variation. Differences between the mean squares were then tested using F statistics. The species predicted to have the higher amount of variation was set as the numerator and that predicted to have the less variability was set as the denominator of the variance ratio. Bonferroni corrections were applied to account for the use of each dataset in two comparisons. Since white-plumed honeyeaters are parasitised most frequently, they are predicted to have less within-clutch variation and more between-clutch variation than dusky woodswallows and willie wagtails. Of these two occasional hosts, willie wagtails have more recorded parasitisms (Table 2.2). To determine if significance is more than we might expect to find by chance, I also tested the opposing hypothesis, that species with a higher rate of parasitism have more within-clutch variation.

In addition to using variance ratios, the effect of degree of parasitism on the amount of colour variability within clutches was analysed using least squares regression (JMP 6.0). The within-clutch variation (as measured by standard deviation) for each of the first three principal components was designated the dependent variable with rate of parasitism the independent variable.

Results:

DISCRIMINATION ABILITIES

Do hosts reject foreign eggs?

Despite some crossing-over of individual eggs' colours into chromatic regions of 'neighbouring' species, DFA clearly separated eggs of different species into spectrally unique groupings (Fig 2.2). This provides rough guidelines for how much contrast in egg colour exists between species (mean squared distances, Table 2.5). All three host species demonstrated an ability to discriminate and reject foreign eggs. White-plumed honeyeaters, the major hosts, were able to reject eggs of all level of contrast, while dusky-woodswallows and willie wagtails, the two occasional hosts, showed poor discrimination of low contrast eggs, but were strong rejectors of high contrast eggs (Table 2.5; Fig. 2.3).

What influences rejection rate?

I first tested whether the day of the nesting cycle in which the experimental egg was added influenced the likelihood of rejection (accept/reject) using a logistic regression. This variable was not significant in either the conservative ($\chi^2_1 < 0.01$, $p = 0.94$) or the full dataset ($\chi^2_1 = 0.70$, $p = 0.40$), so nests parasitised at different times were all included in the following analyses.

Next, I tested whether the independent variables 'egg-matching', 'within-clutch variation', and 'rate of parasitism' influenced the likelihood of rejection using a logistic regression. Three models were fitted, one for each principal component. The only significant variables were the egg-matching scores for PC1 ($\chi^2_1 = 9.24$, $p < 0.01$) and PC2 ($\chi^2_1 = 4.81$, $p = 0.03$), which suggests as degree of difference in brightness (PC1) and reds (PC2) between the host clutch and foreign egg increases, so does rejection

(Fig. 2.4). The conservative dataset gave the same results (PC1: $\chi^2_1 = 7.77$, $p < 0.01$; PC2: $\chi^2_1 = 5.14$, $p = 0.02$).

The effect of parasitism rate on clutch variation

When eggs from the field were analysed using reflectance spectrophotometry, white-plumed honeyeaters and willie wagtails were found to have more between-clutch variation than dusky-woodswallows in all variables measured, with the exception of PC3 for Side (Table 2.6). White-plumed honeyeaters tended to have more between-clutch variation than willie wagtails, but not significantly so.

No measures of within-clutch variation were significant, except PC3 for Side—white-plumed honeyeaters were more variable than dusky woodswallows. PC3 has a strong loading in UV. Overall, the amount of within-clutch variation in these three species was found to be contrary to predictions, and was the same pattern as in between-clutch variation: white-plumed honeyeaters had the most, followed by willie wagtails, and dusky woodswallows had the least (Table 2.7). This was supported by regression analysis, which demonstrated that within-clutch variation (the standard deviation of PC1, PC2, and PC3) increased with higher rates of parasitism. Rate of parasitism increased with within-clutch variation in PC1 ($p < 0.01$), brightness, and PC2 ($p < 0.01$), UV and reds, but not PC3 ($p = 0.20$), which explains UV, blues, and greens (Fig. 2.4); the primary host had more within-clutch variation in PC1 and PC2 and a tendency to be more variable in PC3, while in the occasional hosts willie wagtails had somewhat more variation in PC1 than the less parasitised dusky woodswallows.

Discussion:

This is one of the first studies to show that Australian cuckoo hosts can reject foreign eggs (see Welbergen *et al.* 2001) and the first study to test the extent of discrimination abilities in these three pallid cuckoo hosts. As predicted by coevolutionary theory (Rothstein 1990; Davies 2000), each species was able to reject foreign eggs, and the major host showed a greater ability to discriminate eggs very similar to its own than the occasional hosts. However, the amount of parasitism to which a host species is subject did not influence its rate of rejection. Thus the more frequently parasitised species was better at rejecting eggs that look similar to its own, but did not reject a higher percentage of foreign eggs overall. Rejection is most notably determined by the difference in appearance between the host eggs and the foreign egg in the first two principal components, which represent brightness, regions of UV, and reds–browns.

Contrary to predictions, within-clutch variation was not found to affect rejection. This was not entirely surprising because, also contrary to expectation, within-clutch variation was not reduced in the more heavily parasitised host. This suggests that within-clutch variation has not been subject to selection as a result of cuckoo parasitism, and consequently is perhaps relatively unimportant in the process of egg discrimination.

By contrast, the amount of between-clutch variation within each species supported the hypothesis: variability in clutches between individuals increased with increasing rates of parasitism, and major host species were correspondingly better able to discriminate conspecific eggs. This suggests that heavily parasitised hosts may have experienced selection for a higher level of between-clutch variation because this facilitates detection of mimetic cuckoo eggs.

Though within-clutch variation did not decrease in the major host species, it did, however, increase. A possible explanation for this unexpected result is that the

evolution of reduced within-clutch variation may be constrained by selection for increased between-clutch variation. If selection acts more strongly to drive increasing variation between individuals than to lower variability within clutches, then perhaps the strong selection for mutations that allow an individual's egg to diverge from the population mean might also result in an associated increase in within-clutch variation.

There are several factors, however, that may have affected the significance of the results. The first concern is the issue of small sample size. Experimental sample size was constrained by (i) using only three-egg clutches to prevent biases in variance, (ii) needing to measure all eggs in the clutch, including the foreign egg, with a reflectance spectrophotometer, (iii) parasitism of nests before the experiments were scored. Second, my experiments were interspecific, involving a comparison of three different species. Every species is under a unique set of selection pressure so the levels of clutch variation detected may not necessarily be influenced or affected by brood parasitism. However, the three species investigated are sympatric, so any selection pressures imposed by geography are consequently reduced.

A third possible explanatory factor may be that although each species experiences different rates of parasitism, they are all subject to brood parasitism and therefore selection is likely to drive clutch variation of all three species in the same direction. This might reduce existing differences in amount of clutch variation between the three species. Fourth, pallid cuckoos were observed only at Gungahlin Hill and Campbell Park during the two research seasons. When aware of the presence of a potential parasite, plasticity in host behaviour may allow hosts to attempt to reduce parasitism by increasing rejection rate (Davies & Brooke 1988; Davies *et al.* 1996; Brooke *et al.* 1998; Lindholm & Thomas 2000). The three host species were not exposed to an equal number of experimental trials at each study site, so rejection rates

could be confounded by the presence or absence of adult cuckoos at each location. This is an area that would require additional testing.

In addition, using records of parasitism to estimate amount of parasitism between different species is laden with biases. In particular, species differ vastly in the visibility and accessibility of their nests. Parasitism of species with highly visible and accessible nests is likely to be reported more frequently than parasitism of hosts with cryptic or high nests. For this reason, records of parasitism are best used as rough guides to approximate levels of parasitism and are subject to certain inaccuracies.

The age of a host may also affect the acceptance or rejection of a foreign egg. Age is argued to play an important role if birds learn the appearance of their eggs by imprinting on them and can use memory to reject foreign eggs (Lotem *et al.* 1992; 1995). Since age was unknown (with the exception of two dusky woodswallow pairs), this is a potential limitation to my study. This is of particular concern if an experiment was conducted on a first year breeder who had not yet been able to learn the appearance of her clutch—those individuals would be more likely to accept a foreign egg. However, several studies of other passerines failed to find any relationship between age and rejection behaviour (Stokke *et al.* 1999; Soler *et al.* 2000; Amundsen *et al.* 2002).

Environmental conditions may also shape egg colour expression (Lotem *et al.* 1995; Avilés *et al.* 2007). This occurs not only by influencing the physiological condition and stress of the female but also by changing the available food supply (Jones *et al.* 2003; Avilés *et al.* 2007). However, the process of egg pigmentation remains uncertain (Solomon 1987), and only one known study has tested environmental effects on wild populations—Avilés *et al.* (2007) analysed museum egg collections and used historical records of spring rainfall and temperatures to discover that eggshell colour in reed warblers *Acrocephalus scirpaceus* and their parasite, the common cuckoo, are determined in part by the environment. In addition to affecting egg colour,

environmental stresses may also influence nest abandonment (George *et al.* 1992). This is of particular concern because south-eastern Australia experienced severe drought conditions during the period of this research. However, all three species occurred in the same habitat, so probably experienced similar environmental stresses on eggshell colour. Further, effects of augmented nest abandonment due to poor environmental conditions may be of little consequence since analyses were run twice, once including a 'conservative' estimate of rejection which removed those experiments with uncertain causes of nest abandonment. The results calculated from the two datasets are essentially equivalent, indicating the robustness of the data.

Table 2.1. Body weight, egg size (mean length (range) x mean width (range); *n*) and description of ground-colour and markings of species studied in the field. Pallid cuckoo listed for reference (Higgins 1999; Higgins *et al.* 2001; Beruldsen 2003; Higgins *et al.* 2006)

Species	Body weight	Egg size (mm)	Egg description
Pallid cuckoo	80–85 g	23.9 (20.8–26.2) × 17.4 (15.0–19.6); 126	Fleshy pink, sometimes with a few scattered dots of a darker hue
White-plumed honeyeater	18.5 g	20.5 (19.3–21.8) × 15 (14.2–15.7); 20	Colour varies; most commonly buffy white or delicate pinkish white, marked with distinct roundish spots of pinkish brown, usually more numerous at large end
Fuscous honeyeater	16 g	19.6 (18.3–21.3) × 14.6 (14.0–15.2); 11	Colour varies; deep creamy buff with scattered reddish spots at large end, or pink or pinky brown with a few dark/brown speckles
Willie wagtail	18 g	20.1 (17.5–21.3) × 15.1 (14.2–16); 17	Colour varies; creamy-white to cream, spotted mostly about the larger end, with creamy-brown to light to mid-brown
Dusky woodswallow	35 g	22.7 (21.6–23.8) × 17.1 (16.3–18.0); 15	Dull white to creamy-white to cream, marked towards and over the larger end, though sometimes round centre of egg, or rarely around the small end, with splotches of varying shades of brown and underlying markings of lavender

Table 2.2. Percent of total recorded parasitisms by pallid cuckoos for each host species in the biogeographical region of South East Australia and the Murray-Darling

Total no. of recorded pallid cuckoo parasitisms in this region = 376

Host species	No. of recorded parasitisms	% of all parasitisms
Dusky woodswallow	8	2%
Willie wagtail	20	5%
White-plumed honeyeater	33	9%*

* But note that white-plumed honeyeaters are the most common host within the ACT (Taylor 1992), and therefore probably suffer higher parasitism rates than those reported here.

Table 2.3. Description of study areas with total number of experiments conducted on each species

Study area	Species studied	Number of experiments 2006-7	Number of experiments 2007-8
Campbell Park	White-plumed honeyeater	2	0
	Dusky woodswallow	1	0
	Willie wagtail	4	0
Gungahlin Hill Nature Reserve	White-plumed honeyeater	3	2
	Dusky woodswallow	0	2
	Willie wagtail	1	0
Namadgi National Park	Fuscous honeyeater	0	0
	Dusky woodswallow	11	2
	Willie wagtail	1	0
Lake Ginninderra	White-plumed honeyeater	0	8
	Dusky woodswallow	0	4
	Willie wagtail	0	2

Table 2.4. Clutch size and incubation length (approx. no. days after clutch completion) (Higgins *et al.* 2001; Higgins *et al.* 2006)

	White-plumed honeyeater	Dusky woodswallow	Willie wagtail
Clutch size	2 or 3	3 or 4	3 or 4
Incubation length	13–15	14–16	14

Table 2.5. The contrast in egg colour between conspecific and heterospecific eggs and the number of conspecific and heterospecific eggs accepted and rejected in experimental trials. Host species are shown in rows and the species of the egg added to the nests are shown in columns. No experiments were conducted on fuscous honeyeaters. MSD = mean squared distance taken from discriminant function analysis; represents a numeric mean distance in average chroma of individuals from host species to each foreign species, for both side and ring measurements. Reject = number of eggs from each species that were rejected; numbers in parentheses are from the conservative estimate of rejection. Accept = number of eggs accepted by each host species. Contrast = amount of difference between host clutch and foreign egg

Host species	Foreign species: Willie wagtail	Foreign species: Dusky woodswallow	Foreign species: Fuscous honeyeater	Foreign species: White-plumed honeyeater
Willie wagtail	MSD Side: 6.43 MSD Ring: 5.81 Reject: 0 Accept: 8 Contrast: Low	MSD Side: 8.40 MSD Ring: 15.00 Reject: 1 Accept: 1 Contrast: Medium	MSD Side: 22.23 MSD Ring: 41.40 Reject: N/A Accept: N/A Contrast: N/A	MSD Side: 28.79 MSD Ring: 45.25 Reject: 4 Accept: 0 Contrast: High
Dusky woodswallow	MSD Side: 4.95 MSD Ring: 11.12 Reject: 2 (1) Accept: 0 Contrast: Medium	MSD Side:2.98 MSD Ring: 1.93 Reject: 2 (1) Accept: 12 Contrast: Low	MSD Side: 18.70 MSD Ring: 17.15 Reject: N/A Accept: N/A Contrast: N/A	MSD Side: 28.81 MSD Ring: 25.59 Reject: 4 (2) Accept: 0 Contrast: High
Fuscous honeyeater	MSD Side: 19.39 MSD Ring: 44.02 Reject: N/A Accept: N/A Contrast: N/A	MSD Side: 19.31 MSD Ring: 23.65 Reject: N/A Accept: N/A Contrast: N/A	MSD Side: 3.59 MSD Ring: 8.43 Reject: N/A Accept: N/A Contrast: N/A	MSD Side: 9.56 MSD Ring: 12.73 Reject: N/A Accept: N/A Contrast: N/A
White-plumed honeyeater	MSD Side: 29.43 MSD Ring: 47.81 Reject: 2 Accept: 0 Contrast: High	MSD Side: 32.91 MSD Ring: 32.03 Reject: 1 Accept: 1 Contrast: Hight	MSD Side: 13.05 MSD Ring: 12.67 Reject: 3 (2) Accept: 0 Contrast: Medium	MSD Side: 7.07 MSD Ring: 8.37 Reject: 4 (1) Accept: 4 Contrast: Low

Table 2.6. Differences between species in the amount of between-clutch variation for the first three principal components. Due to the use of each dataset in two independent tests, significance was assessed with a Bonferroni correction ($p < 0.025$). Willie wagtails are predicted to have marginally more between-clutch variation and less within-clutch variation than dusky woodswallows. DWS: Dusky woodswallow, WW: Willie wagtail, WPH: White-plumed honeyeater

DWS vs. WPH: $F_{8,16}$			
Ring	P-Value	Side	P-Value
PC1	0.056	PC1	**<0.001**
PC2	**<0.001**	PC2	**<0.001**
PC3	**0.013**	PC3	0.963

WW vs. WPH: $F_{8,6}$			
Ring	P-Value	Side	P-Value
PC1	0.460	PC1	0.064
PC2	0.194	PC2	0.266
PC3	0.439	PC3	0.932

DWS vs. WW: $F_{6,16}$			
Ring	P-Value	Side	P-Value
PC1	0.044	PC1	0.040
PC2	**<0.001**	PC2	**<0.001**
PC3	**0.011**	PC3	0.165

Table 2.7. Differences between species in the amount of within-clutch variation for the first three principal components. Due to the use of each dataset in two independent tests, significance was assessed with a Bonferroni correction ($p < 0.025$). Variables significant in the predicted direction are shown in bold, and variables significant in the opposite direction are marked with asterisks. Willie wagtails are predicted to have marginally more between-clutch variation and less within-clutch variation than dusky woodswallows. DWS: Dusky woodswallow, WW: Willie wagtail, WPH: White-plumed honeyeater

DWS vs. WPH: $F_{34,12}$

Ring	P-Value	Side	P-Value
PC1	1.000*	PC1	0.999*
PC2	1.000*	PC2	0.880
PC3	0.931	PC3	**0.019**

WW vs. WPH: $F_{16,12}$

Ring	P-Value	Side	P-Value
PC1	0.952	PC1	0.993*
PC2	0.999*	PC2	0.266
PC3	0.911	PC3	0.629

DWS vs. WW: $F_{30,16}$

Ring	P-Value	Side	P-Value
PC1	0.982*	PC1	0.915
PC2	0.661	PC2	0.930
PC3	0.791	PC3	0.855

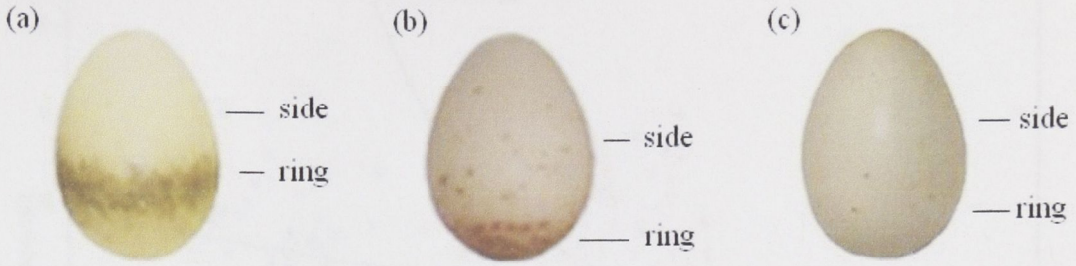
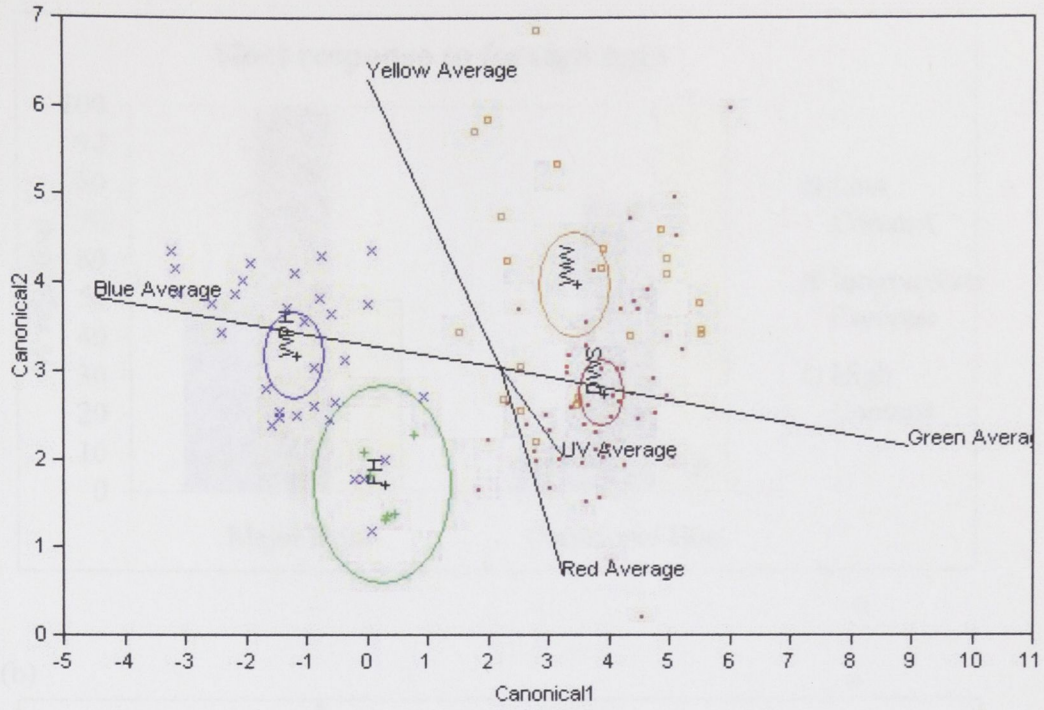


Figure 2.1. Points of measurement, Side and Ring, for reflectance spectrophotometry readings. (a) Ring measured at halo of speckles (e.g. willie wagtail eggs); (b) Ring measured at darker hue near base (e.g. yellow-tufted honeyeater eggs); (c) nearly immaculate egg, Ring measured near base (e.g. pallid cuckoo eggs)

(a)



(b)

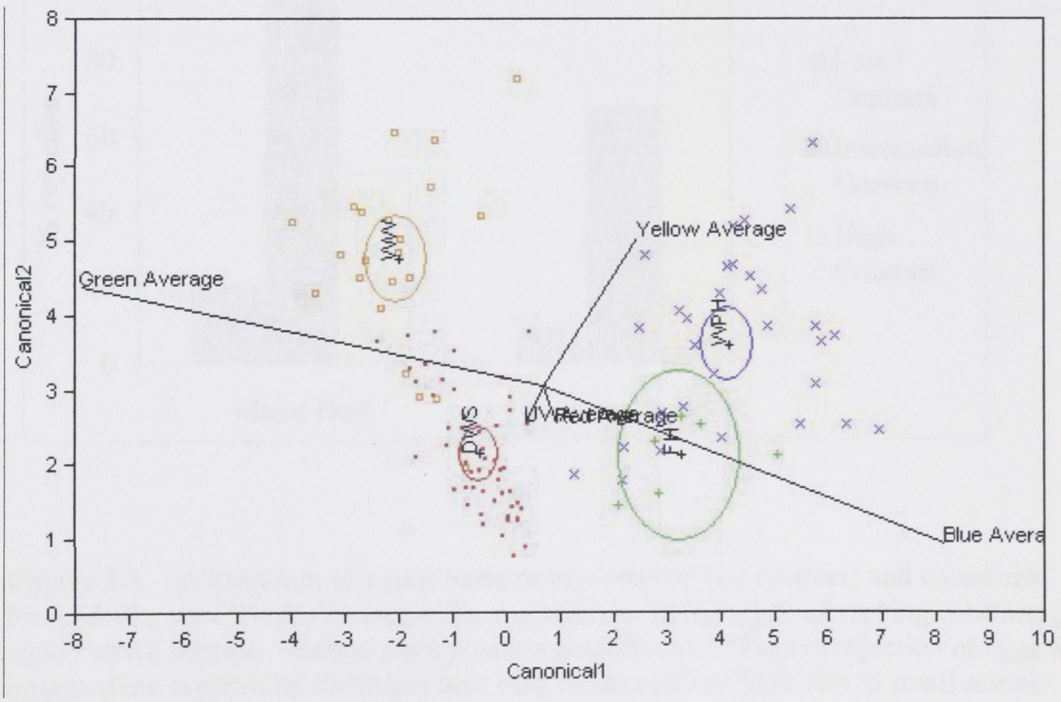
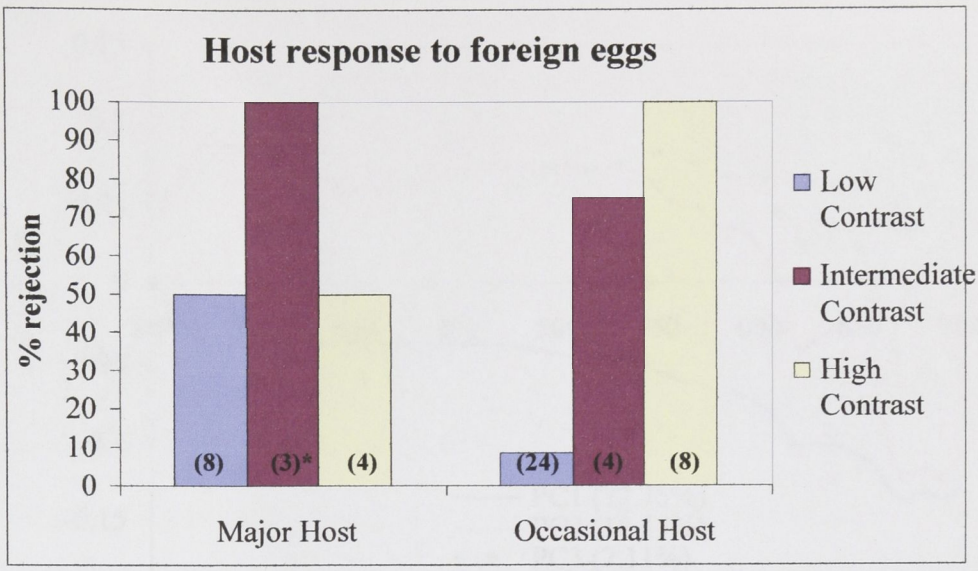


Figure 2.2. Discriminant function analysis of willie wagtail (WW, *n* = 22), dusky woodswallow (DWS, *n* = 53), white-plumed honeyeater (WPH, *n* = 31), and fuscous honeyeater (FH, *n* = 6) eggs from the field. (a) Side; (b) Ring. Discriminant function analysis labels each multivariate mean with a circle, and the size of the circle corresponds to a 95% confidence limit for the mean. Groups that are significantly different have non-intersecting circles

(a)



(b)

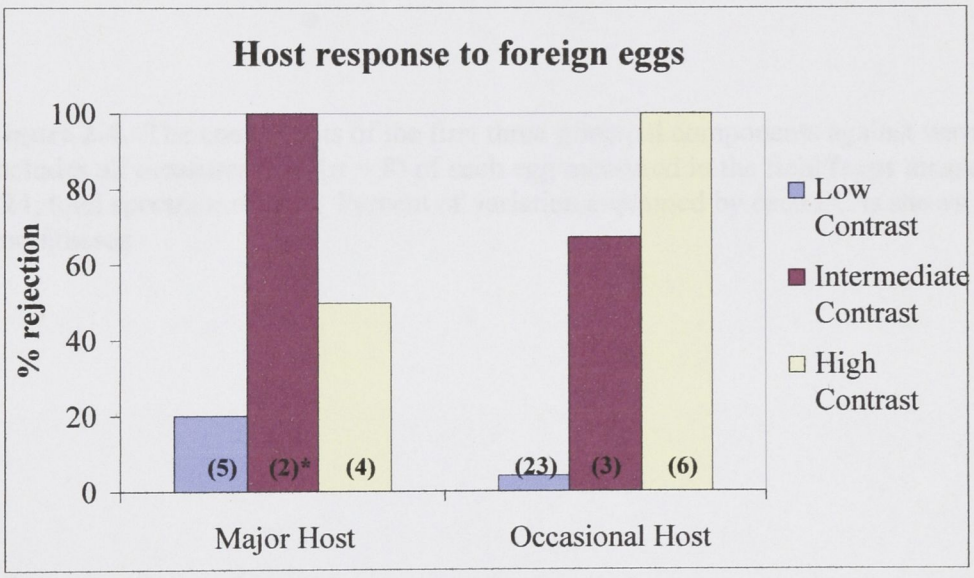


Figure 2.3. (a) Response of major hosts (white-plumed honeyeaters) and occasional hosts (dusky woodswallows and willie wagtails) to foreign eggs of varying contrast; (b) conservative dataset. Sample sizes given in parentheses. *Percent rejection of eggs of intermediate contrast by the major host may be deceptively high due to small sample size

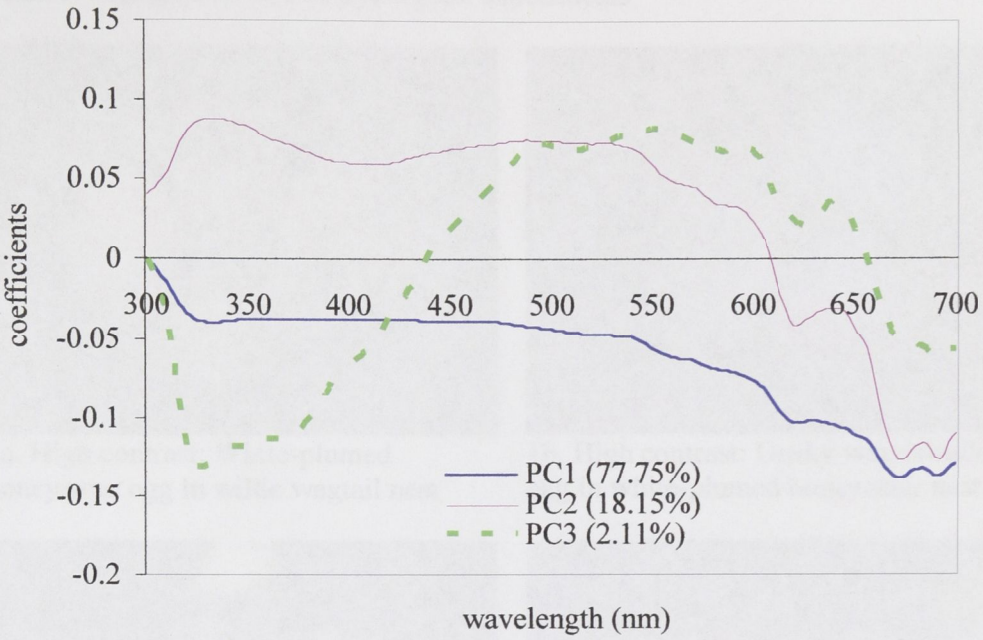


Figure 2.4. The coefficients of the first three principal components against wavelength; includes all measurements (n = 8) of each egg measured in the field (eggs measured n = 124, total spectra n = 992). Percent of variation explained by each PC is shown in parentheses

Plate 1. Examples of nest manipulation experiments

1a. High contrast: White-plumed honeyeater egg in willie wagtail nest

1b. High contrast: Dusky woodswallow egg in white-plumed honeyeater nest

1c. Intermediate contrast: Dusky woodswallow egg in willie wagtail nest

1e. Intermediate contrast: Fuscous honeyeater egg in white-plumed honeyeater nest

1f. Low contrast: Willie wagtail egg in willie wagtail nest

1g. Low contrast: Dusky woodswallow egg in dusky woodswallow nest

1e. Low contrast: White-plumed honeyeater egg with white-plumed honeyeater eggs

Chapter 3:

Does clutch variation differ between populations that experience different rates of parasitism?

Introduction:

The debate over clutch variation initiated in Chapter 2 is expanded here by encompassing a much wider geographic range. Using data collected from eggs stored in Australian museum collections, I investigate how rate of parasitism affects egg colouration and clutch variation by comparing a species that is heavily parasitised in one part of its range but rarely parasitised in another. As in previous intraspecific studies that compared the differences in variation between parasitised and non-parasitised populations (see Chapter 1: Table 1.2, Experiment Type 2), I compare a heavily parasitised subspecies with a lesser-parasitised subspecies of three pallid cuckoo hosts: Singing honeyeaters *Lichenostomus virescens*, red wattlebirds *Anthochaera carunculata*, and yellow-throated miners *Manorina flavigula.* I also compare two fairly evenly parasitised subspecies of yellow-tufted honeyeaters *Lichenostomus melanops* to act as a control.

The strength of this examination lies in not only analysing multiple species, but also in comparing populations of the same species. Interspecific comparisons (see Chapter 1: Table 1.1) fail to compensate for the variety of evolutionary pressures faced by different species, which may affect clutch variation in ways that are unrelated to parasitism. In addition, my analysis is not biased by the human visual system because eggshell colour was measured quantitatively using reflectance spectrophotometry.

I test three hypotheses about the impact of parasitism on eggshell colouration. First, I predict that the overall egg colour in a population will change more rapidly with increasing rates of parasitism, because any genetic mutations that alter individuals' egg colour will provide those individuals with an advantage in detecting a cuckoo egg. Therefore, I expect to find a greater difference in egg colouration between populations in those species that differ in parasitism rate than in those with equivalent parasitism rates between populations. Next, I continue the investigation from Chapter 2 of

determining whether parasitism has driven the evolution of high between-clutch variability and low within-clutch variation by comparing subspecies that are under greater parasitism pressures than their less frequently parasitised counterparts.

Methods:

SPECIES SELECTION

Four host species were selected for analysis on the basis of their geographically variable parasitism rate: yellow-tufted honeyeaters, singing honeyeaters, yellow-throated miners, and red wattlebirds. Again, rates of parasitism were based on the large-scale survey conducted by Brooker and Brooker (1989), as detailed in Chapter 2. The issues of species-specific biases in records of parasitism revealed in Chapter 2 are avoided here, because the analyses compare different populations of the same species, not different species. Due to this, each species warrants separate rather than combined analysis.

Brooker and Brooker (1989) provide records of parasitism for individual biogeographical regions within Australia (based on Blakers *et al.* 1984), which allow a relative approximation of the amount of parasitism of geographically separated host populations. As in Chapter 2, the relative rate of parasitism for each population was estimated by dividing the number of parasitisms by pallid cuckoos for each population by the total number of parasitisms for all species in their respective biogeographical region (Table 3.1). Both subspecies of yellow-tufted honeyeater are located within the same region, so the number of recorded parasitisms for each population was determined by using localities provided by the Australian Cuckoo-Host Database (Brooker & Brooker 2005) and assigning subspecies to the clutches based on known subspecies distribution within the region.

Three hosts of pallid cuckoos, all of which are meliphagid honeyeaters, show marked geographical variability in patterns of parasitism by pallid cuckoos (Brooker & Brooker 1989): singing honeyeaters are found throughout most of the country, but the south-western subspecies is most commonly parasitised. Yellow-throated miners are prevalent across most of Australia and are common hosts in the west while less parasitised in the east. Red wattlebirds occur in southern Australia and are major hosts in the south-west, but not in the south-east. None of these hosts are listed as major hosts of any other species of cuckoo (Brooker & Brooker 1989), although in the eastern ranges north of Sydney red wattlebirds are parasitised by the larger Australian koel, *Eudynamys scolopacea* (Brooker & Brooker 1989; Davies 2000). Because of this parasitism by a different species of cuckoo, and the potential for conflicting selection pressures on egg colour, red wattlebird clutches from Queensland were excluded from the analyses.

Though each of the chosen species has more than two geographically separated subspecies, I chose for comparison only the most parasitised subspecies and the least parasitised subspecies. A fourth species, the yellow-tufted honeyeater, was used as a control species because the subspecies appear to be equally parasitised (Brooker & Brooker 1989).

MUSEUM COLLECTIONS

Egg clutches selected for colour analysis were provided by CSIRO's Australian National Wildlife Collection in Canberra, the Australian Museum Oological Collection in Sydney, and the Department of Terrestrial Vertebrates at the Western Australian Museum in Perth. CSIRO held a large number of eggs collected from across Australia while the Australian Museum and Western Australian Museum supplemented the

sample size with clutches primarily from eastern Australia and western Australia, respectively.

Information from the museum databases were used to determine locality and the date the clutches were collected. Only complete clutches with clear labelling were used. Also, clutches that contained either dirty or heavily cracked eggs were disregarded, since these conditions may give inaccurate reflectance readings.

Eggshell colour was measured using a reflectance spectrophotometer, following the methods from Chapter 2. The spectrophotometer was re-standardised after every fifth clutch.

STATISTICAL ANALYSIS

Spectra means plots

To plot and compare the average reflectance spectra of the two subspecies of each species, I first determined the mean spectrum across all measurements made on each egg for both points of measure, Ring (n = 4) and Side (n = 4). The mean spectrum (for both Ring and Side, separately) of each subspecies was then obtained by averaging the spectra (300–700 nm) of all the eggs within each subspecies. The plot of the mean spectrum shows the spectrogram curves and illustrates the colours of the eggs of the two subspecies.

A summary plot including standard error was also made for Ring and Side for each subspecies by separating the mean spectra into 50 nm segments the entire length of the spectrum (300–700 nm). Each segment was averaged to include 25 points, one taken every 2 nm. The mean of these segments were then found for each subspecies, and the standard error was calculated.

Differences between preserved eggs and fresh eggs

To determine potential differences in colouration between preserved museum eggs and fresh eggs, I combined the plots of mean spectrum (for both Ring and Side) of all dusky woodswallow, white-plumed honeyeater, and willie wagtail eggs measured in the field, separately for each species (data taken from Chapter 2), with samples of preserved eggs of the same species collected from within the Australian Capital Territory and the surrounding regions of New South Wales. The most recently collected eggs were used in order to reduce the effects of age on colouration.

Principal Components Analysis

The rest of my analyses make use of principal components analysis. The first three principal components (PC1, PC2, PC3) were determined for both points of measure—from the individual egg averages of Ring and Side—separately for each species so as to avoid characteristics and complications unique to each species. Both subspecies were included in the same analysis for each species.

Are there differences in eggshell colour between subspecies?

Though the spectra means plots give a visual idea of the colour reflectance of each subspecies, to find any statistically significant differences in the spectrum between two subspecies of the same species, I used the score of PC1, PC2, and PC3 for both Ring and Side measurements as the dependent variable in linear mixed models (REML). The age of the eggs (in years) and the subspecies they belong to were entered as fixed effects to determine if these influenced the PC scores. Only eggs of known age were included in the analysis. I also examined possible interactions between these variables (age and subspecies), and the least significant terms were dropped so only significant terms remained. The eggs in each clutch were given the same nest identifier and these

identifiers were set as the random variable to account for repeated measures, since there was typically more than one egg per clutch.

Are there differences in clutch variation between subspecies?

To examine possible differences between subspecies in the amount of between-clutch and within-clutch variations in egg appearance, I followed the statistical methods from Chapter 2 (*The effect of parasitism rate on clutch variation*) and entered each PC score (separately for Ring and Side) into a general linear model. Included in the models were age (using the date when the clutch was collected) and subspecies. Clutches with unknown age were not included, due to limitations of the model. For the control species, the ratio was arbitrarily placed so that either subspecies was predicted to have 'less within-clutch' and 'more between-clutch' variation than the other. Significance in either direction was tested. All analyses in this chapter were conducted using GenStat10.

Results:

PRINCIPAL COMPONENTS PLOT SUMMARY

The first three principal components explained between 98.4 and 99.6 % of the total variance in spectrum shape for the ring and the side of each species (ranges: PC1 85.0–92.33%, PC2 3.9–9.2%, PC3 3.01–5.5%; n = 8). The coefficients of each principal component were plotted to illustrate the major sources of variation in the spectra they represent (Figure 3.2).

ARE MUSUEM EGGS DIFFERENT IN COLOURATION FROM FRESH EGGS?

Plotting the mean reflectance spectra of museum eggs with fresh eggs for each species shows preserved museum eggs are brighter across the entire spectrum than fresh eggs from the field (Fig. 3.3). Though preserved eggs are older, this difference in colouration is not a result of age, since age does not tend to affect PC1 (Table 3.2), which typically represents brightness (Fig. 3.2).

ARE THERE DIFFERENCES IN CLUTCH APPEARANCE BETWEEN SUBSPECIES?

The plots of mean spectra show the average reflectance for the two points of measure—Ring and Side—for both subspecies of each species (Figure 3.1). This gives a summary of how the egg colour differs at the two points of measure, as well as how the eggshells of the geographically separated subspecies are different. The difference between Ring and Side is consistent—Side is brighter than Ring. Brightness is indicated by the overall 'height' of the spectrum and is a measure of reflectance intensity (Endler 1990). This disparity in brightness between the two points is representative of the often dark and heavy speckling that characterises the Ring region (Fig 3.1, left column). There are also dissimilarities between the subspecies of each species, again most distinctly in brightness (Fig 3.1, left column). In general, the shapes of the spectra are similar between subspecies, suggesting similarities in chroma (purity of a colour, characterised by steepness of the slope in a spectrum) and hue (the wavelength of the maximum reflectance) (Endler 1990). The summary plots, which show standard error, reflect these similarities and differences (Fig. 3.1, right column).

To determine how the two subspecies are statistically different in eggshell colour, I ran linear mixed models including both terms (age of the clutches and subspecies) and the interaction. There was no interaction in any of the models, so this term was dropped in order to obtain the minimal model (age and subspecies). While the

age of the eggs did have an effect on colour in every species, most strongly in PC2, there were also significant differences in colour between populations. However, species that showed the greatest disparity in parasitism rate were no more different between populations than the species with more similar parasitism rates (Table 3.2).

DOES RATE OF PARASITISM AFFECT WITHIN-CLUTCH VARIATION?

In the three species with contrasting parasitism rates between populations (red wattlebirds, yellow-throated miners, and singing honeyeaters) I examined whether the variation within clutches was lower in the more parasitised populations. Of 18 total variables tested (PC1, PC2, PC3 for Ring and Side) in the three experimental species, three showed a correlation in the predicted direction (Table 3.3). To determine whether this number of significant variables is more than we might expect to find by chance, I also tested the opposing hypothesis, that subspecies with a higher rate of parasitism have more within-clutch variation. No variables were significant (Table 3.3).

IS BETWEEN-CLTUCH VARIATION AFFECTED BY RATE OF PARASITISM?

I next examined if populations with greater parasitism have more between-individual variation. In the three experimental species, one variable was significant in the predicted direction, one was significant in the opposite direction, and 16 showed no correlation between rate of parasitism and between-individual clutch variability (Table 3.4).

DOES CLUTCH VARIATION DIFFER BETWEEN TWO POPULATIONS WITH SIMILAR RATES OF PARASITISM?

In the control species, both subspecies are evenly parasitised, so they were predicted to have equal amounts of variation within their clutches and between

individuals. However, two out of 12 variables, both for within-clutch variation, were significantly different between the two populations.

Discussion:

POPULATION DIFFERENCES IN EGG COLOUR

In the never-ending arms race between cuckoos and their hosts, we might expect that parasitism would select for ever-changing host egg colour so as to evade egg mimicry by cuckoos. If so, egg colour should change at a faster rate in heavily parasitised than less parasitised populations. In other words, egg morphology would diverge more rapidly between populations with increasing disparity in parasitism rate between populations. I found little support for this, which suggests parasitism does not cause large-scale changes in colour in host populations.

Alternatively, this could be a result of the small sample size of *L. v. virescens* (n = 8 clutches) and *L. m. cassidix* (n = 5); these two populations had the smallest sample sizes of all the subspecies analysed, and any possible variations in colour could have been undetected.

POPULATION DIFFERENCES IN CLUTCH VARIABILITY

Differences in parasitism rate between populations of pallid cuckoo hosts had a modest influence on within-clutch egg variability; all three host species showed significantly lower within-clutch variation in the more heavily parasitised subspecies. Differences in between-clutch variation, however, were less significant—two species showed significant differences in PC2, yet one was in the predicted direction and one in the opposite.

However, not only were these differences generally only evident in one of the six measures of within-clutch and between-clutch variation for each species, but they were only weakly significant and were not consistently related to any particular feature of egg colour. Further, the control species, which was predicted to show the same degree of clutch variation in the two populations, also showed some significant differences in within-clutch variation. All these caveats suggest that effect of parasitism rate on clutch variability is not strong in these species.

These rather weak results might be explained by the fact that all the subspecies are parasitised, though at varying degrees. Even uncommon parasitism provides some pressure for hosts to develop defensive abilities, particularly since the outcome of successful parasitism is so devastating to a host's reproductive fitness (Langmore *et al.* 2005). Consequently, heavily parasitised and occasionally parasitised populations both experience selection in the same direction, so differences in clutch variation may be very slight.

Two previous studies have also utilised reflectance spectrophotometry to measure clutch variation by comparing two populations of the same host species—one population being sympatric with cuckoos, the other allopatric and totally non-parasitised (Table 1.2, Experiment Type 2). Avilés and Møller (2003) found within-clutch variability in the UV to decrease in populations of host species living with parasitic cuckoos (between-clutch variation was not analysed), and Lahti's findings (2005) supported the hypothesis for both within-clutch and between-clutch variation. These two studies found strong correlations, yet they included a totally non-parasitised comparison population. If selection is acting to alter clutch variability, by comparing a sympatric population with an allopatric population it would be expected these studies would have more significant results than by comparing two parasitised populations.

MUSEUM EFFECTS

To effectively use preserved eggs from private and public collections, it is first necessary to understand their potential biases and differences from fresh eggs discovered in the field. Museum holdings are excellent resources for vast amounts of readily available data, such as information on species distributions and taxonomy, and can provide snapshots of ecological shifts and changes (Brooke 2000). However, preserved specimens are not the same as if they had been fresh from the field. As in a similar study involving stored eggs (Starling *et al.* 2006), I found that eggshell colour changed with age. This change occurred most notably in PC2, which typically explains 300–340 and 600–660 nm wavelengths. In only one instance did age affect PC1 (brightness). This suggests the overall vibrancy of the eggshells remained consistent with time, but was subject to some changes in UV and reds/browns. This supports evidence from previous studies (eg. Starling *et al.* 2006) that it is necessary to control for the age of museum clutches in studies of egg colour.

There was no interaction between age and subspecies, and since the museums tended to hold clutches primarily of one subspecies of each species (the Western Australian Museum in Perth supplying the majority of the western *M. f. obscura* subspecies while CSIRO in Canberra providing the bulk of the eastern *M. f. flavigula* clutches, for example) it can be argued there was no difference between the ways the museums were handling their eggs; old eggs were in similar condition at each of the museums.

In addition to the effects of age, stored eggs are also subject to changes caused by the preservation process; it is standard for collectors to empty the contents of an egg, leaving only a hollow shell. When a sample of preserved willie wagtail, dusky woodswallow, and white-plumed honeyeater clutches collected from south-eastern Australia was compared with the clutches measured in the field—which were all from

south-eastern Australia—the museum clutches were much brighter along the entire spectrum than the fresh eggs. This brightness could be caused by the light from the spectrophotometer probe reflecting inside the hollow eggs and illuminating them, or from external light entering into the hollow eggs more easily than the solid eggs. It is important to be aware of this difference between the two types of eggs, especially when using preserved eggs to represent those found in the field.

Overall, these analyses detected weak support for decreased within-clutch variation as a result of pallid cuckoo parasitism, and no support for changes in egg-shell colour or amount of between-clutch variation. However, these results could be made more conclusive with increased sample size, though all usable clutches from the three primary collections were measured and included in this study.

Table 3.1. Records of parasitism for two subspecies of each host species from Brooker & Brooker (1989). Number of recorded parasitisms for subspecies in region / Total number of recorded parasitisms in region = Percent of total regional pallid cuckoo parasitisms for each subspecies. *n* = Total no. of clutches measured for this study. SW (South West); SE (South East) grouped with MD (Murray-Darling); (Brooker & Brooker 1989)

Red wattlebird	*Carunculata*: SE, MD 11 / 376 3% *n* = 37	*Woodwardi:* SW 42 / 139 30% *n* = 41
Singing honeyeater	*Sonorus:* SE, MD 4 / 376 1% *n* = 45	*Virescens*: SW 17 / 139 12% *n* = 18
Yellow-throated miner	*Flavigula*: SE, MD 5 / 376 1% *n* = 57	*Obscura:* SW 17 / 139 12% *n* = 42
Yellow-tufted honeyeater	*Cassidix*: SE, MD 10 / 376 3% *n* = 10	*Meltoni:* SE, MD 9 / 376 3% *n* = 41

Table 3.2. The effect of age and subspecies on egg colour, represented by the first three principal components (PC1, PC2, PC3) for Ring and Side measurements. Significant results are highlighted in bold. Species listed in putative order of parasitism disparity, from red wattlebirds (highest) to yellow-tufted honeyeaters (little/none)

		Ring			Side		
	Fixed term	F statistic	Degrees of freedom	F pr	F statistic	Degrees of freedom	F pr
Red wattlebird							
PC1	Subspecies	5.27	1, 28	**0.029**	2.40	1, 29	0.132
	Age	5.59	1, 29	**0.025**	0.59	1, 29	0.449
PC2	Subspecies	3.19	1, 28	0.085	1.04	1, 29	0.316
	Age	6.17	1, 28	**0.019**	6.58	1, 29	**0.016**
PC3	Subspecies	0.22	1, 29	0.644	0.01	1, 29	0.912
	Age	7.86	1, 29	**0.009**	0.95	1, 29	0.339
Yellow-throated miner							
PC1	Subspecies	1.05	1, 27	0.316	8.45	1, 27	**0.007**
	Age	0.04	1, 27	0.836	0.21	1, 27	0.654
PC2	Subspecies	9.82	1, 27	**0.004**	1.84	1, 27	0.186
	Age	14.02	1, 27	**<0.001**	10.00	1, 27	**0.004**
PC3	Subspecies	11.07	1, 27	**0.003**	14.06	1, 27	**<0.001**
	Age	2.07	1, 27	0.162	1.24	1, 27	0.274
Singing honeyeater							
PC1	Subspecies	1.09	1, 20	0.308	0.55	1, 21	0.466
	Age	0.28	1, 20	0.605	0.67	1, 20	0.424
PC2	Subspecies	0.95	1, 20	0.342	0.64	1, 20	0.432
	Age	9.16	1, 20	**0.007**	5.10	1, 20	0.035
PC3	Subspecies	2.90	1, 21	0.104	1.60	1, 20	0.220
	Age	1.87	1, 20	0.186	1.49	1, 20	0.236
Yellow-tufted honeyeater							
PC1	Subspecies	2.20	1, 22	0.152	4.12	1, 22	0.055
	Age	2.92	1, 22	0.101	0.56	1, 22	0.463
PC2	Subspecies	5.53	1, 22	**0.028**	1.03	1, 22	0.323
	Age	9.48	1, 22	**0.005**	8.01	1, 22	**0.010**
PC3	Subspecies	0.01	1, 22	0.905	2.20	1, 22	0.152
	Age	5.07	1, 22	**0.035**	0.14	1, 22	0.716

Table 3.3. Differences between subspecies in the amount of within-clutch variation for the first three principal components. Significant variables are in bold; variables significant in the opposing direction (ie. the more highly parasitised subspecies has more within-clutch variation than the less parasitised subspecies) are marked with asterisks. Species listed in putative order of parasitism disparity, from red wattlebirds (highest) to yellow-tufted honeyeaters (little/none)

Red wattlebird: $F_{21,13}$			
Ring	P-Value	Side	P-Value
PC1	0.930	PC1	0.941
PC2	0.667	PC2	0.817
PC3	**0.035**	PC3	0.853

Yellow-throated miner: $F_{42,27}$			
Ring	P-Value	Side	P-Value
PC1	0.058	PC1	**0.037**
PC2	0.400	PC2	0.508
PC3	0.441	PC3	0.422

Singing honeyeater: $F_{26,5}$			
Ring	P-Value	Side	P-Value
PC1	0.531	PC1	0.630
PC2	0.154	PC2	**0.026**
PC3	0.654	PC3	0.055

Yellow-tufted honeyeater: $F_{22,4}$			
Ring	P-Value	Side	P-Value
PC1	0.561	PC1	0.866
PC2	0.907	PC2	**0.999***
PC3	**0.989***	PC3	0.765

Table 3.4. Differences between subspecies in the amount of between-clutch variation for the first three principal components. Significant variables are marked in bold; variables significant in the opposing direction (ie. the more highly parasitised subspecies has less between-clutch variation than the less parasitised subspecies) are marked with asterisks. Species listed in putative order of parasitism disparity, from red wattlebirds (highest) to yellow-tufted honeyeaters (little/none)

Red wattlebird: $F_{12,17}$

Ring	P-Value	Side	P-Value
PC1	0.073	PC1	0.078
PC2	0.946	PC2	0.901
PC3	0.708	PC3	0.649

Yellow-throated miner: $F_{10,17}$

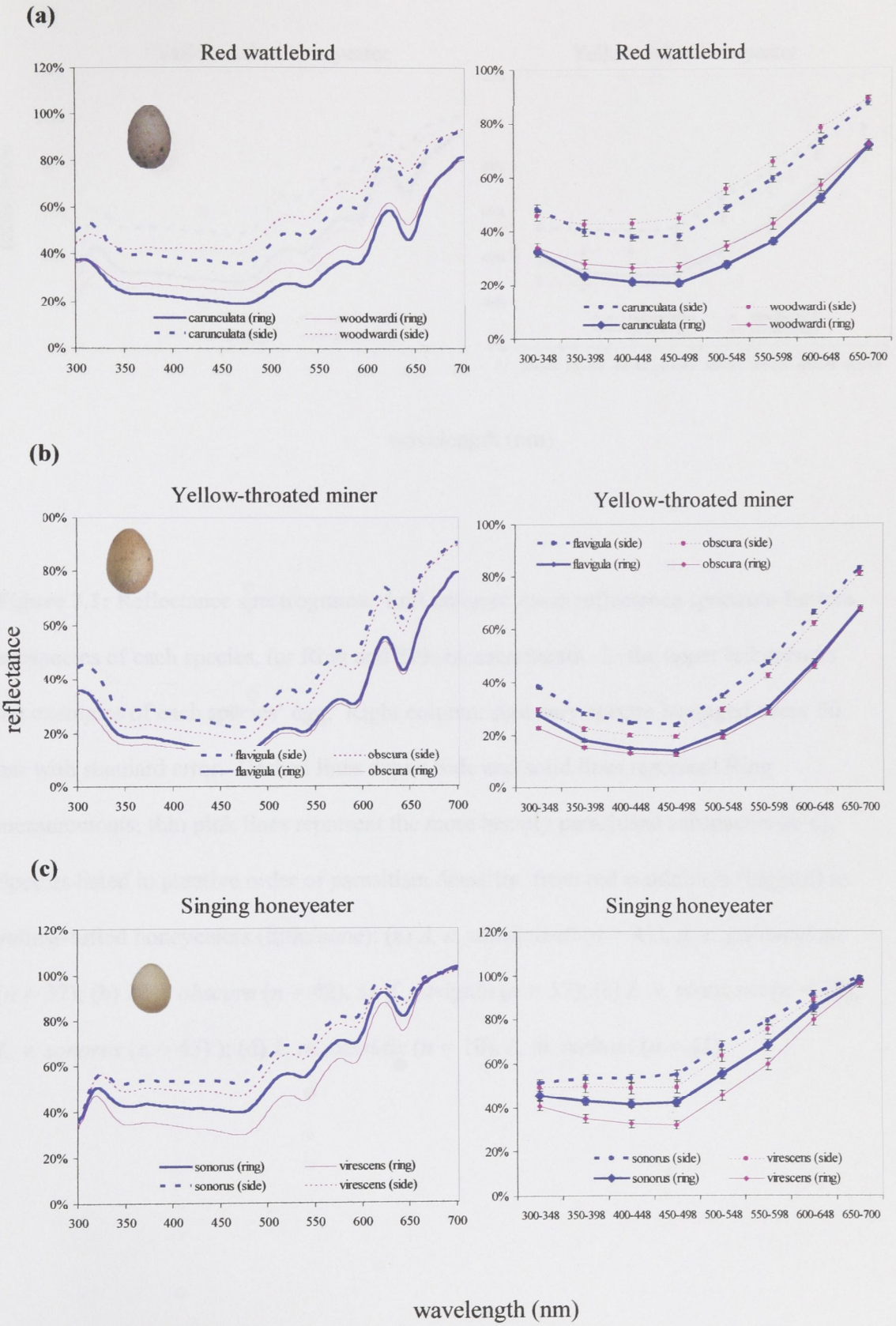
Ring	P-Value	Side	P-Value
PC1	0.907	PC1	0.461
PC2	**0.049**	PC2	0.627
PC3	0.226	PC3	0.863

Singing honeyeater: $F_{4,16}$

Ring	P-Value	Side	P-Value
PC1	0.888	PC1	0.597
PC2	**0.979***	PC2	0.904
PC3	0.840	PC3	0.294

Yellow-tufted honeyeater: $F_{3,19}$

Ring	P-Value	Side	P-Value
PC1	0.385	PC1	0.842
PC2	0.866	PC2	0.915
PC3	0.615	PC3	0.931


(a)
Red wattlebird
120%
100%
80%
60%
40%
20%
0%
300 350 400 450 500 550 600 650
carunculata (ring)
carunculata (side)
woodwardi (ring)
woodwardi (side)
Red wattlebird
100%
80%
60%
40%
20%
0%
carunculata (side)
carunculata (ring)
woodwardi (side)
woodwardi (ring)
300-348 350-398 400-448 450-498 500-548 550-598 600-648 650-700
(b)
Yellow-throated miner
reflectance
00%
80%
60%
40%
20%
0%
300 350 400 450 500 550 600 650 700
flavigula (side)
flavigula (ring)
obscura (side)
obscura (ring)
Yellow-throated miner
100%
80%
60%
40%
20%
0%
flavigula (side)
flavigula (ring)
obscura (side)
obscura (ring)
300-348 350-398 400-448 450-498 500-548 550-598 600-648 650-700
(c)
Singing honeyeater
120%
100%
80%
60%
40%
20%
0%
300 350 400 450 500 550 600 650 700
sonorus (ring)
sonorus (side)
virescens (ring)
virescens (side)
Singing honeyeater
120%
100%
80%
60%
40%
20%
0%
sonorus (side)
sonorus (ring)
virescens (side)
virescens (ring)
300-348 350-398 400-448 450-498 500-548 550-598 600-648 650-700
wavelength (nm)

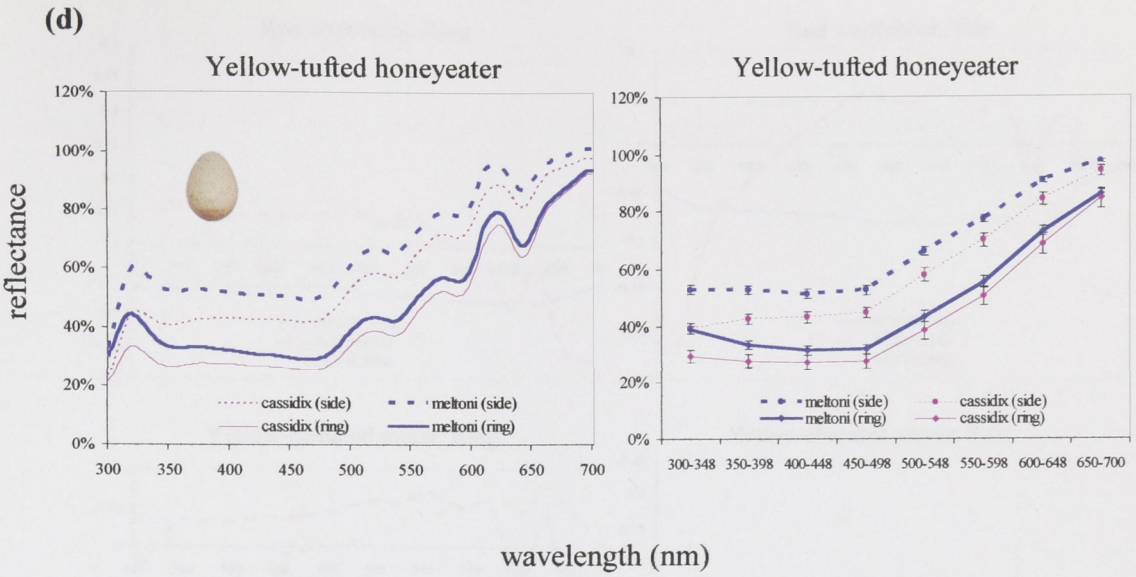


Figure 3.1: Reflectance spectrograms. Left column: mean reflectance spectrum for two subspecies of each species, for Ring and Side measurements. In the upper left corners are examples of each species' egg. Right column: summary spectra averaged every 50 nm with standard error. Dashed lines depict Side and solid lines represent Ring measurements; thin pink lines represent the more heavily parasitised subspecies (a–c). Species listed in putative order of parasitism disparity, from red wattlebirds (highest) to yellow-tufted honeyeaters (little/none); (a) *A. c. woodwardi* (n = 41), *A. c. carunculata* (n = 37); (b) *M. f. obscura* (n = 42), *M. f. flavigula* (n = 57); (c) *L. v. virescens* (n = 18), *L. v. sonorus* (n = 45)); (d) *L. m. cassidix* (n = 10), *L. m. meltoni* (n = 41)

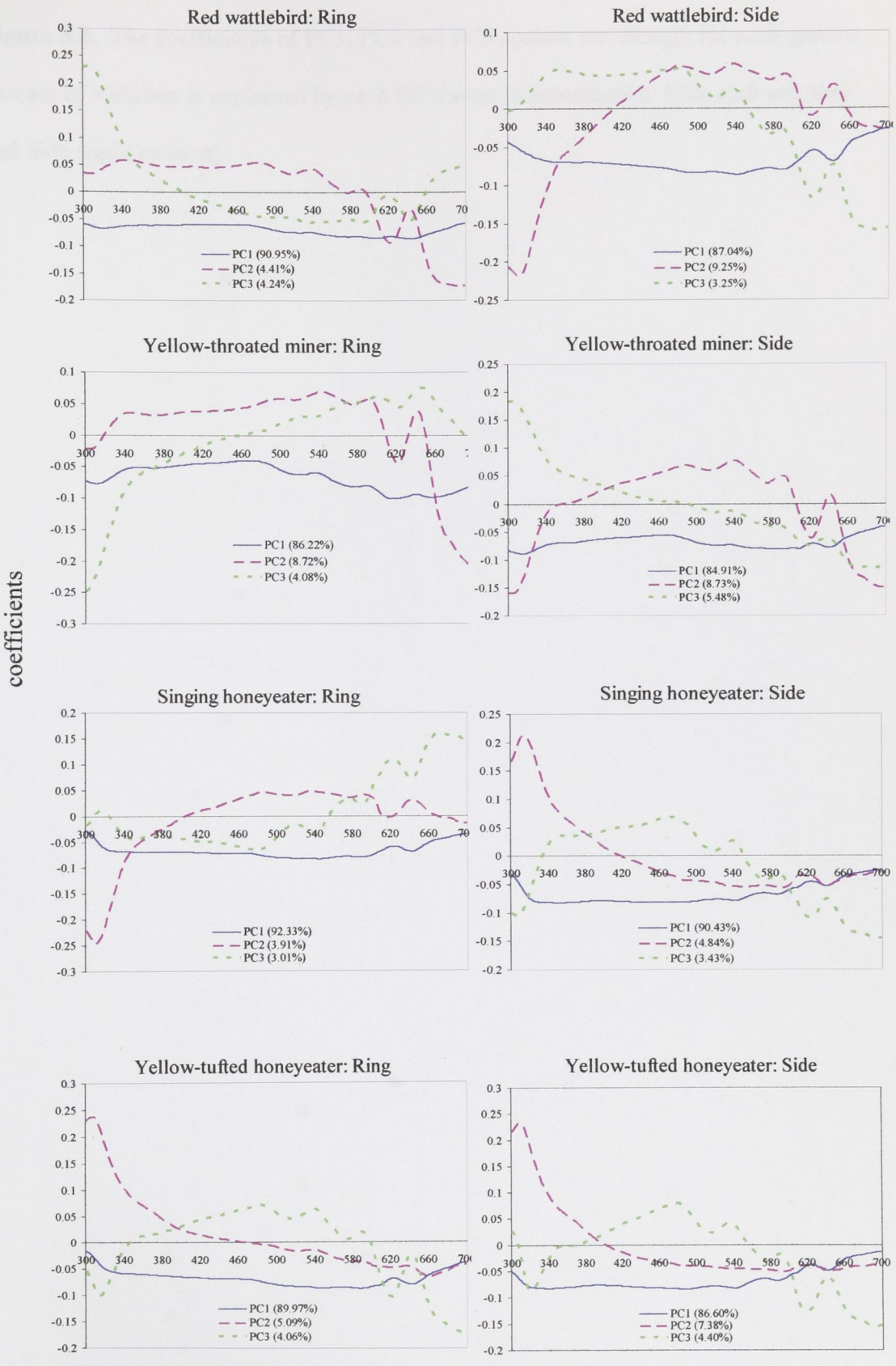

Red wattlebird: Ring
PC1 (90.95%)
PC2 (4.41%)
PC3 (4.24%)
Red wattlebird: Side
PC1 (87.04%)
PC2 (9.25%)
PC3 (3.25%)
Yellow-throated miner: Ring
PC1 (86.22%)
PC2 (8.72%)
PC3 (4.08%)
Yellow-throated miner: Side
PC1 (84.91%)
PC2 (8.73%)
PC3 (5.48%)
Singing honeyeater: Ring
PC1 (92.33%)
PC2 (3.91%)
PC3 (3.01%)
Singing honeyeater: Side
PC1 (90.43%)
PC2 (4.84%)
PC3 (3.43%)
Yellow-tufted honeyeater: Ring
PC1 (89.97%)
PC2 (5.09%)
PC3 (4.06%)
Yellow-tufted honeyeater: Side
PC1 (86.60%)
PC2 (7.38%)
PC3 (4.40%)
coefficients
wavelength (nm)

Figure 3.2. The coefficients of PC1, PC2 and PC3 against wavelength for each species. Percent of variation is explained by each PC shown in parentheses. Ring (left column) and Side (right column)

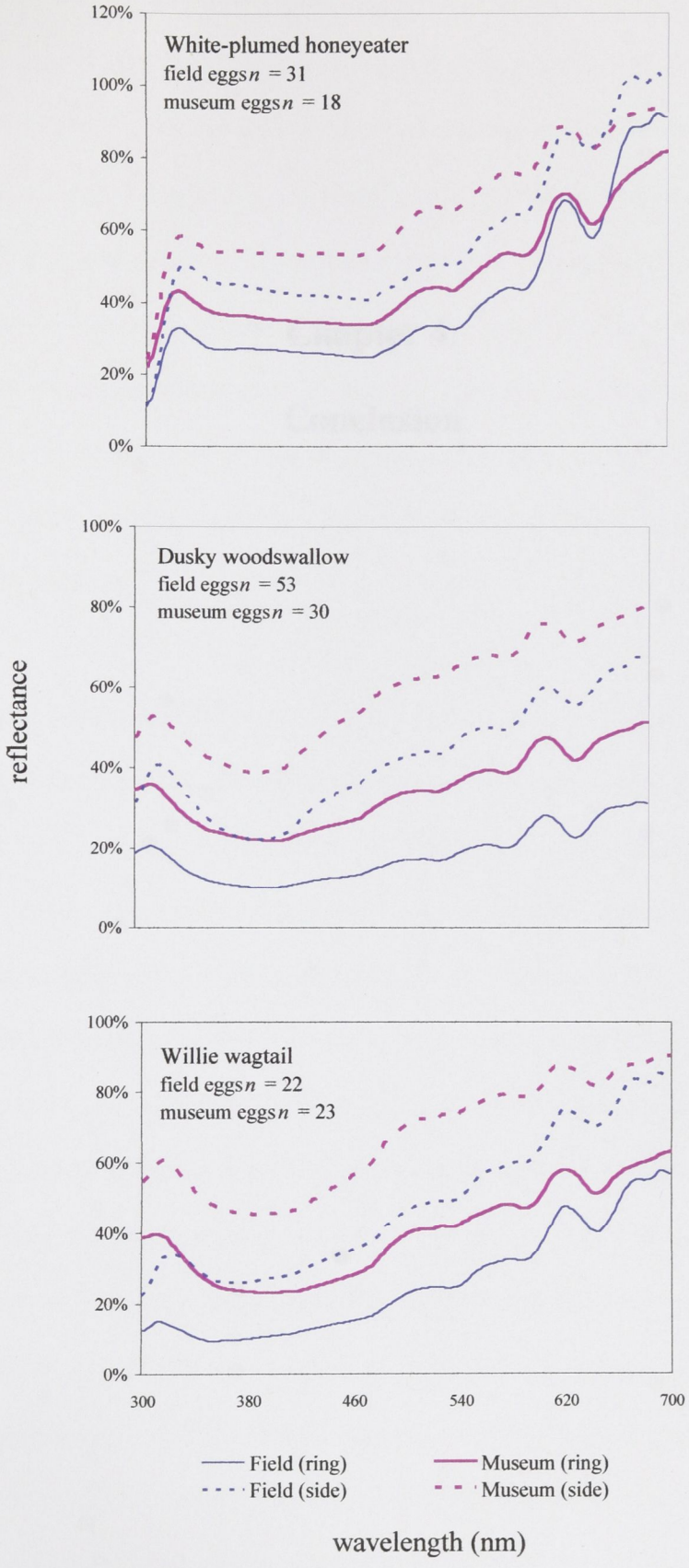


Figure 3.3: Mean reflectance spectra of field eggs and local museum eggs

Chapter 4:

Conclusion

Brood parasitism provides one of the best examples of a coevolutionary arms race (Rothstein *et al.* 2002): the reciprocal exchange in adaptations between parasite and host advances each party in an attempt to outmanoeuvre the other, with many cuckoos laying highly mimetic eggs and hosts showing impressive powers of egg discrimination. The primary intention of this study was to address the question of whether the coevolutionary arms race has progressed one step further—to the modification of host clutches so as to impede mimicry by cuckoos and assist egg discrimination by hosts. Overall I found mixed support for this possibility, and in general my results corresponded with those of other studies examining this question. I tested four hypotheses:

1. Does reduced within-clutch variation facilitate egg discrimination?

In opposition to original predictions, within-clutch variation was not found to influence the rate of foreign egg rejection. This supports the growing number of intraspecific studies that have measured the amount of within-clutch variation of rejectors versus acceptors in a host population (Table 1.2, Expt. Type 1). Of the eight studies that were based on human vision to calculate clutch variation, six did not detect a significant difference in within-clutch variation between those hosts that rejected foreign eggs and those that accepted (Procházka & Honza 2003; Honza *et al.* 2004; Lovászi & Moskát 2004; Procházka & Honza 2004; Stokke *et al.* 2004; Antonov *et al.* 2006). Moreover, two of the three studies that used reflectance spectrophotometry contradicted the original hypothesis and found within-clutch variation to be higher, not lower, in rejectors (Avilés *et al.* 2004; Cherry *et al.* 2007). One argument for why intraclutch variation may actually increase as a result of parasitism relies on the memory of the host, as well as the degree of contrast of the parasitic egg. If a host indeed can accurately remember several eggs in its nest, it may be easier to pick out any cuckoo

eggs if her own eggs are variable. Even if the cuckoo can closely mimic the eggs of the species as a whole, it would have difficulty mimicking all the eggs in any one clutch (Cherry *et al.* 2007).

The over-arching consensus of these studies suggests that lower variability in appearance of eggs within a clutch does not aid in the recognition and rejection of a parasitic egg.

2. Have higher parasitism rates selected for reduced within-clutch variation?

Given the conclusion (above), that reduced within-clutch variation does not appear to improve a host's ability to detect a foreign egg, it is not surprising that I found little support for the hypothesis that higher parasitism rates select for reduced within-clutch variation. When comparing two populations of the same host species, variation within clutches tended to be lower in the more frequently parasitised populations. However, significant differences in within-clutch variation were also found between two populations of the control species, casting doubt on the argument that these differences were due to differences in parasitism rate, rather than just occurring by chance. Further, when comparing different species, within-clutch variation was actually *higher* in the more frequently parasitised species.

3. Does increased contrast between host and foreign eggs facilitate egg discrimination?

The degree of contrast between host clutch and foreign egg was found to be an important factor in determining probability of rejection. This is supported by previous intraspecific studies (Procházka & Honza 2003; Honza *et al.* 2004; Lovászi & Moskát 2004; Stokke *et al.* 2004; Antonov *et al.* 2006), which, like this study, not only found no influence of amount of within-clutch variation on egg rejection, but also found that the degree of contrast was a reliable indicator of egg rejection. These studies found less

mimetic eggs to be rejected at a higher frequency, yet there was no difference in amount of within-clutch variability between those hosts that accepted and those that rejected the foreign egg.

In most cases where degree of contrast is more important than within-clutch variation, the species under examination has naturally high between-clutch variation (Procházka & Honza 2003; Honza *et al.* 2004; Lovászi & Moskát 2004; Stokke *et al.* 2004). According to the original hypothesis, if hosts in a population lay clutches that look different from each other, it is it harder for cuckoos to mimic the eggs of any particular individual (Swynnerton 1918; Davies & Brooke 1989b; Honza *et al.* 2004; Kilner 2006). Therefore, high between-clutch variation is an advanced adaptation for hosts to make even the most 'mimetic' cuckoo eggs contrast with their own. This is supported by evidence that, like other birds, cuckoo egg appearance is genetically determined, so females lay the same egg type throughout their entire lives (Baldamus 1892, Rey 1892, as cited in Jourdain 1925; Collias 1993). This means that cuckoos cannot preferentially lay eggs to match their host's clutch. In addition, brood parasites are evolutionarily selected to lay eggs that are closest to the mean host egg appearance (Takasu 2003). When a cuckoo lays an egg in any particular clutch, the amount of contrast between the parasitic egg and the host clutch depends on the amount of between-clutch variation in the population (Kilner 2006). In summary, high between-clutch variation increases contrast between individual host clutches and parasitic eggs, which in turn helps hosts identify and reject the foreign eggs.

4. Does increased parasitism increase between-clutch variation?

The conclusion (above) that a high degree of contrast between the host and foreign egg facilitates egg discrimination, would lead us to expect to find a significant effect of parasitism rate on between-clutch variation. However, I found mixed support.

Comparison of two geographically isolated populations of the same host species provided no support for the hypothesis that between-clutch variation is higher in the more heavily parasitised population. Yet at the species level, between-individual variation did increase with higher amounts of parasitism.

Significance of results in relation to degree of comparison

As the degree of comparison increases, from within-population, to between-population, to between-species, the overall significance of differences in clutch variation increases. This is evident in both this study and in previous studies.

The within-population level compares the amount of clutch variation of individuals who accept foreign eggs with those who reject. In the field, within-clutch variation was not significantly correlated with rejection rate, though increased contrast between foreign egg and host clutch facilitated rejection. These findings are supported by previous studies (Table 1.2, Expt. Type 1).

The difference in clutch variability at the population level was measured using museum collections and compared populations of the same host species that were parasitised to varying degrees. This provided weak support for the hypothesis that parasitism decreases within-clutch variation and little support for an increase in between-clutch variation. This result was comparable to previous studies that compared host species allopatric or sympatric to cuckoos, which also suggest some support for the predicted hypotheses (Table 1.2, Expt. Type 2). As a whole, these earlier studies are in somewhat stronger support of both hypotheses than the present study. This may be due to them comparing a totally non-parasitised population with a parasitised population. This study, however, used two parasitised populations, in which case the significance of the results may be diminished by the same directional selection acting on both populations, though to differing degrees. Further, the parasitism rates of different

populations may vary over time, such that populations with currently low rates of parasitism may have been parasitised heavily in the past. This could result in high between-clutch variation and low within-clutch variation persisting despite reduced parasitism, if clutch modification does not carry a cost.

The most significant results are drawn from the largest degree of comparison—between-species tests. In the field, this was measured by comparing three species with differing rates of parasitism. As parasitism increased between the species, within-clutch and between-clutch variation increased. Past examinations, which have compared suitable host species that are parasitised with unsuitable, non-parasitised species (Table 1.1), are supported by the current study and showed little significance for a reduction in within-clutch variation in those species that are parasitised, but more conclusive evidence for an increase in between-clutch variation. The difference in within-clutch variation between my field study and previous studies may be a result of the fact that all species in this study were parasitised, whilst the previous studies used completely non-parasitised populations for comparison.

In general, when looking at the experiments based on order of increasing degree of comparison, it becomes evident that increased between-clutch variation is more strongly selected for than reduced within-clutch variation. Any changes in within-clutch variation may even be constrained by the increase in between-clutch variation. This is supported by a comparative study by Kilner (2006), which suggests hosts are better able to reject foreign eggs by increasing between-clutch variation rather than by lowering variation within their own clutches.

High levels of clutch variation between individuals may lead to the formation of discrete polymorphisms in host eggs, and eventually polymorphic cuckoo eggs (Takasu 2003). The African village weaverbird *Ploceus cucullatus* is a model example of extreme, genetically determined, egg polymorphisms that are theorised to result from

brood parasitism—parasitism from conspecifics and/or from the diederik cuckoo *Chrysococcyx caprius* (Victoria 1972; Collias 1993).

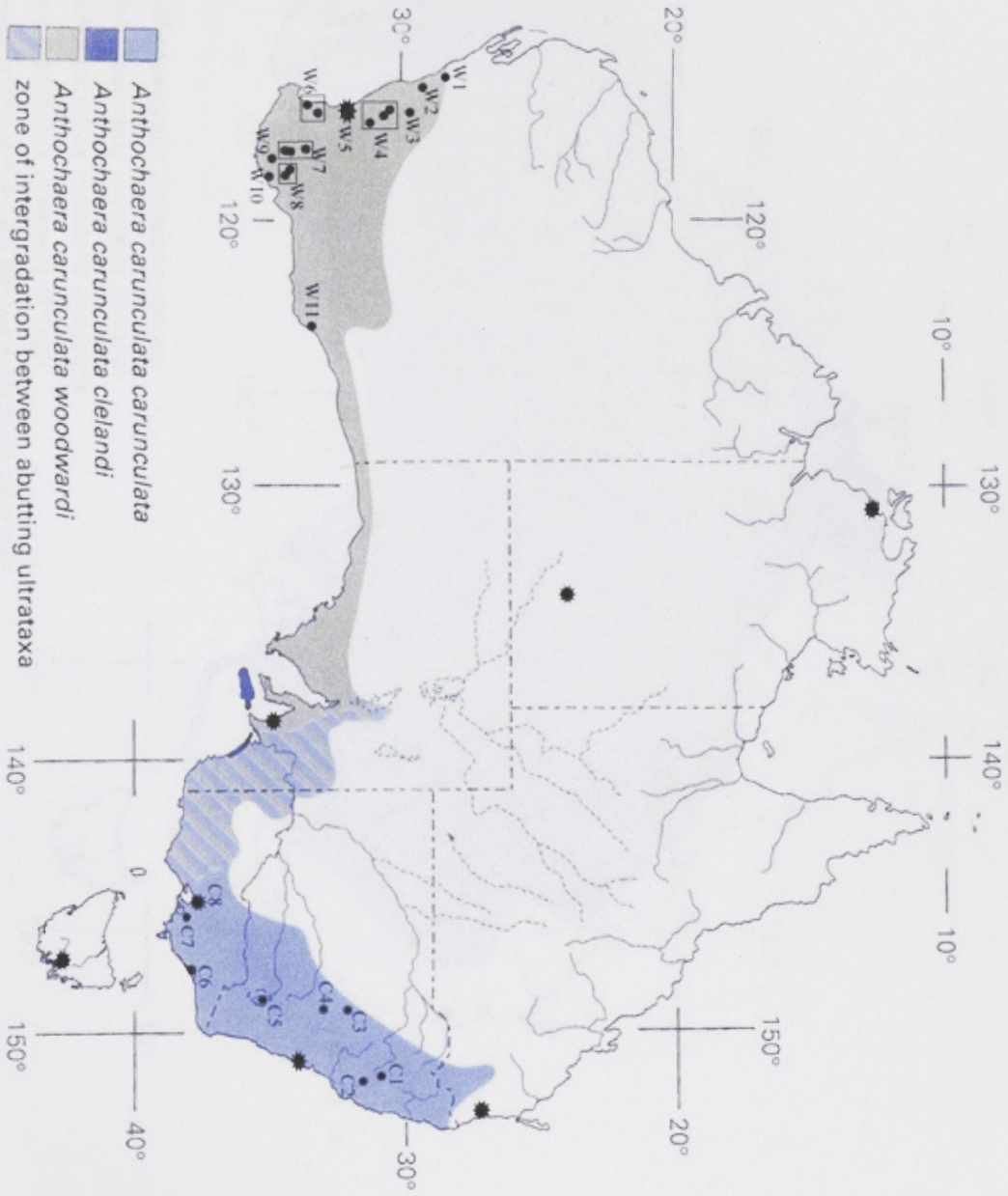
Future studies

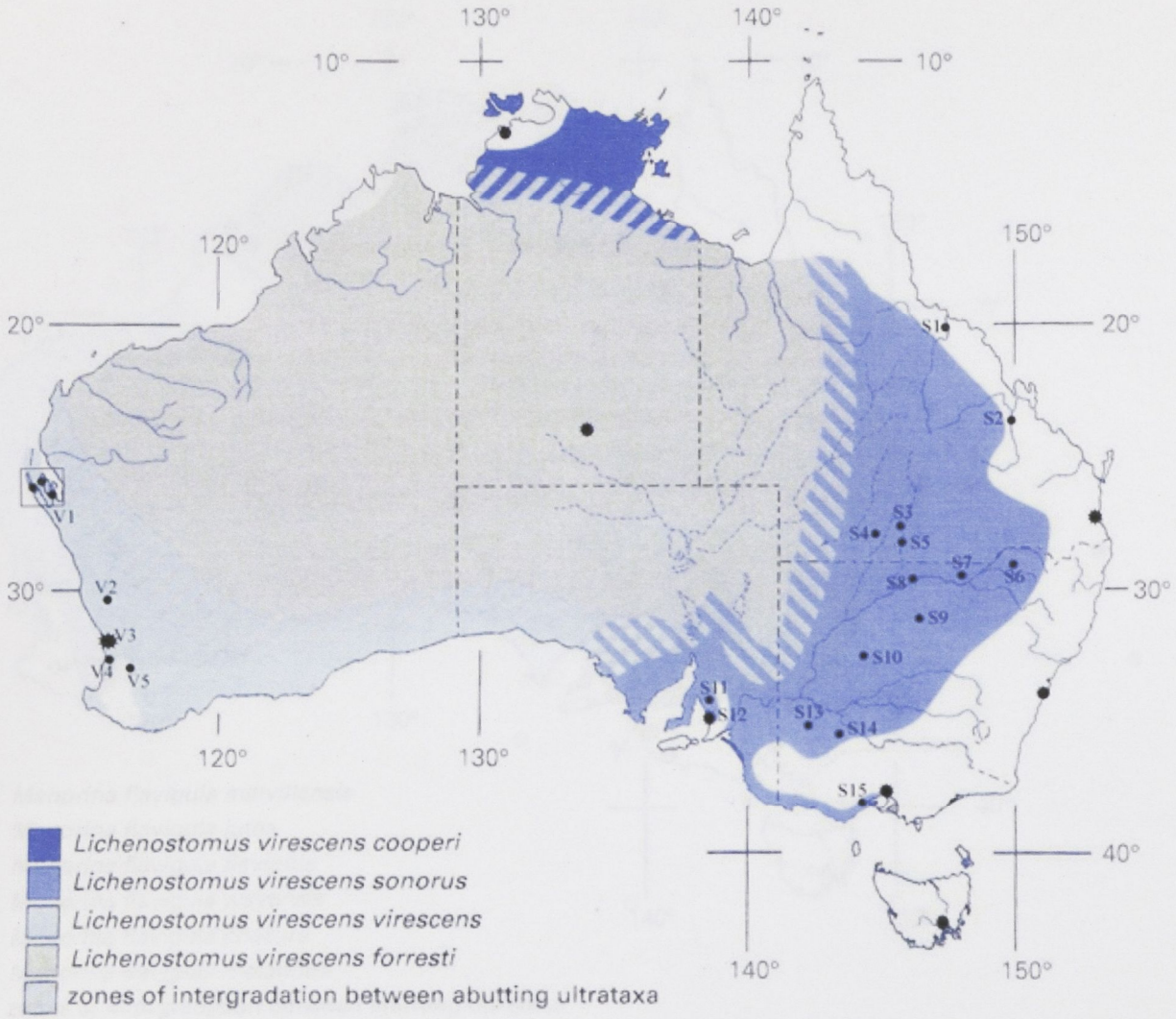
In the field, much can be discovered about a specific host's abilities to recognise and reject foreign eggs if the age of the host is known. By banding birds, individuals who are known to have breeding experiences can be used to test these hypotheses and reduce potential biases due to the use of naive females who have not yet learned their own egg type. It might also be informative to add a highly non-mimetic blue egg to the nest of first-time breeders to determine if they could identify the foreign egg without first learning the appearance of their own clutch. These same hosts could be tested in multiple years to determine if they consistently accept or reject the blue egg. However, it would be necessary to test whether only the female of the species, and not the mate, rejects foreign eggs, and if so, then only the female need be banded and tested.

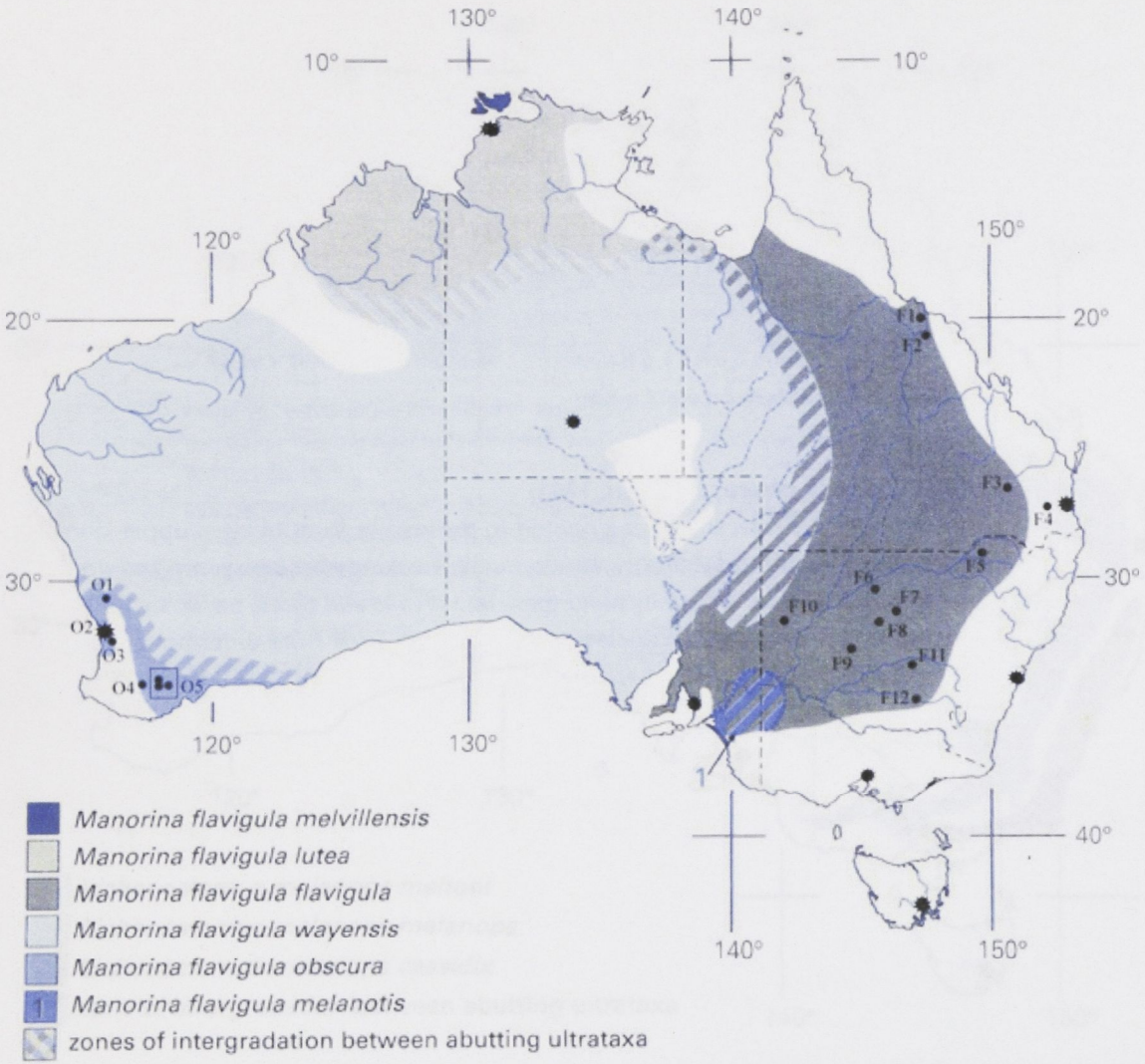
For future experiments to detect the greatest amount of difference in clutch variation, there are several conditions that should be met. First, any comparative study (between-species or between-population) would need to involve a greater knowledge of actual rates of parasitism and ideally knowledge of both past and present rates. Second, experiments should compare host populations allopatric and sympatric with cuckoos. This would be problematic for pallid cuckoo hosts, because to my knowledge, no populations completely evade parasitism. The introduction of host species to parasite-free locations (or alternatively the adoption of a recently-introduced new host species by a cuckoo) would provide the opportunity to meet these requirements, because the timing of the introduction is usually known, so changes in clutch variation after loss of parasitism can be tracked accurately over time. For all experiments, the use of a reflectance spectrophotometer is valuable in not only quantifying colour, but also in

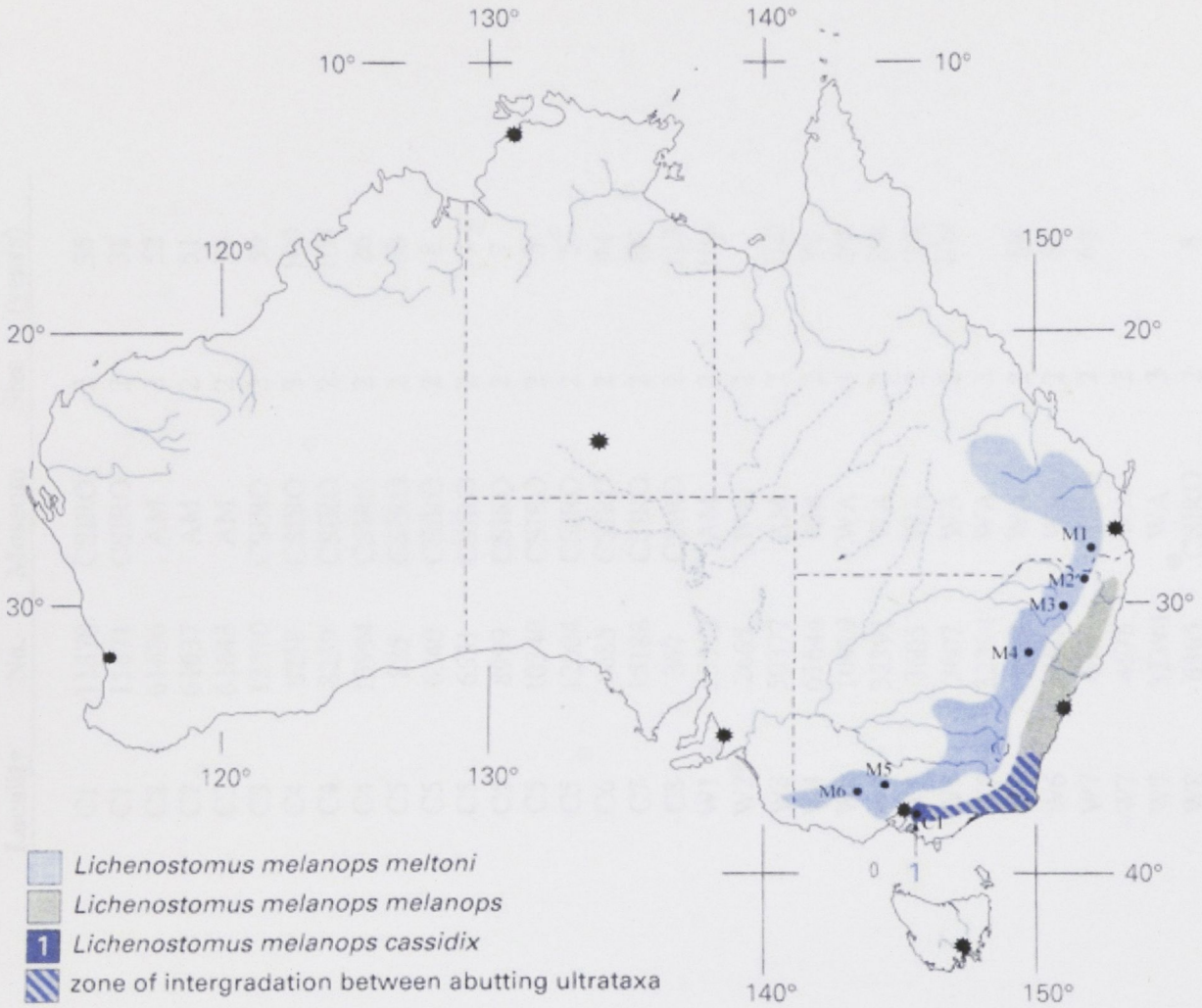
measuring colours invisible to humans but detected by birds. However, quantification of clutch variability could be improved with the use of a computer program that could accurately quantify the size, density and distribution of maculation. If these conditions can be met, it could potentially confirm if higher between-clutch variation is more important in hampering mimicry by cuckoos than reduced within-clutch variation is in rejecting foreign eggs. If so, then it would be exciting to determine if variability within-clutches correspondingly increases with greater amounts of between-individual variation. Such a study would substantially enhance our understanding of this advanced stage of coevolution between cuckoos and their hosts.

APPENDIX A: Distribution maps of pallid cuckoo host species analysed in Chapter 3. Maps reproduced from the *Directory of Australian Birds: Passerines* (Schodde & Mason 1999) with permission from the authors. Specimen localities identified and listed in the following table.




130°
140°
10°
10°
120°
150°
20°
20°
30°
30°
120°
130°
140°
150°
40°
S1
S2
S3
S4
S5
S6
S7
S8
S9
S10
S11
S12
S13
S14
S15
V1
V2
V3
V4
V5
Lichenostomus virescens cooperi
Lichenostomus virescens sonorus
Lichenostomus virescens virescens
Lichenostomus virescens forresti
zones of intergradation between abutting ultrataxa


130°
140°
10°
10°
120°
150°
20°
20°
30°
30°
O1
O2
O3
O4
O5
F1
F2
F3
F4
F5
F6
F7
F8
F9
F10
F11
F12
1
120°
130°
140°
150°
40°
Manorina flavigula melvillensis
Manorina flavigula lutea
Manorina flavigula flavigula
Manorina flavigula wayensis
Manorina flavigula obscura
Manorina flavigula melanotis
zones of intergradation between abutting ultrataxa


130°
140°
10°
10°
120°
150°
20°
20°
30°
30°
M1
M2
M3
M4
M5
M6
C1
120°
130°
140°
150°
0
1
40°
Lichenostomus melanops meltoni
Lichenostomus melanops melanops
1 Lichenostomus melanops cassidix
zone of intergradation between abutting ultrataxa

Red wattlebird

Locality	Reg. No.	Museum	Clutch Size	Age (years)
C1	11870	CSIRO	2	35
C1	11871	CSIRO	2	32
C2	61636	AM	2	52
C2	61637	AM	2	51
C2	61643	AM	2	33
C3	13370	CSIRO	2	59
C4	8258	CSIRO	3	100
C4	8259	CSIRO	2	109
C4	13994	CSIRO	2	26
C5	825	CSIRO	2	53
C5	6340	CSIRO	2	8
C5	6551	CSIRO	2	7
C5	8999	CSIRO	2	7
C5	10340	CSIRO	2	5
C5	12004	CSIRO	2	5
C6	8685	CSIRO	2	94
C7	15188	CSIRO	2	86
C8	367	CSIRO	2	118
W1	55512	AM	2	104
W2	2665	WA	2	
W3	50377	AM	2	100
W4	61644	AM	2	92
W4	10979	WA	3	90
W4	32391	WA	2	104
W5	3605	WA	2	108
W5	3607	WA	2	108
W5	32394	WA	3	
W6	1822	WA	2	88
W6	13606	WA	2	86
W7	8088	CSIRO	2	68
W7	4578	WA	2	
W7	32390	WA	3	
W8	6365	CSIRO	2	8
W8	3061	WA	2	
W9	50378	AM	2	95
W10	2076	CSIRO	2	95
W11	20019	WA	2	22

Singing honeyeater

Locality	Reg. No.	Museum	Clutch Size	Age (years)
S1	12773	CSIRO	2	46
S2	9981	CSIRO	2	94
S3	12774	CSIRO	2	31
S4	69494	AM	2	52
S5	69495	AM	2	53
S6	36582	AM	2	
S7	55806	AM	2	100
S8	50300	AM	2	97
S9	13931	CSIRO	3	26
S9	13932	CSIRO	2	26
S9	13933	CSIRO	3	31
S10	8216	CSIRO	3	109
S10	19523	AM	2	
S11	11057	AM	3	109
S11	66968	AM	2	101
S12	24849	AM	3	108
S13	6476	CSIRO	3	47
S14	66969	AM	2	96
S15	9885	CSIRO	3	101
V1	11771	CSIRO	2	31
V1	66972	AM	2	87
V1	4746	WA	3	
V1	26025	WA	2	13
V2	66971	AM	3	89
V3	3595	WA	2	100
V4	2037	WA	2	
V5	23102	AM	2	18

Yellow-throated miner

Locality	Reg. No.	Museum	Clutch Size	Age (years)
F1	11838	CSIRO	3	32
F1	12828	CSIRO	4	43
F2	12829	CSIRO	3	46
F2	12830	CSIRO	4	46
F2	12831	CSIRO	3	46
F3	5108	CSIRO	3	77
F4	588	CSIRO	3	70
F5	364	CSIRO	3	87
F6	11837	CSIRO	3	35
F7	11836	CSIRO	3	45
F8	2084	CSIRO	3	96
F8	13966	CSIRO	3	39
F8	13967	CSIRO	3	39
F9	8238	CSIRO	4	99
F10	363	CSIRO	3	99
F10	9936	CSIRO	3	100
F11	482	CSIRO	3	37
F12	5099	CSIRO	3	41
O1	1842	WA	4	87
O1	10972	WA	4	92
O1	32368	WA	4	73
O1	32369	WA	4	73
O2	55441	AM	3	107
O2	3597	WA	3	100
O3	20757	WA	3	77
O4	5113	CSIRO	3	69
O4	57738	AM	3	69
O5	11834	CSIRO	3	57
O5	11835	CSIRO	4	52
O5	32384	WA	4	99

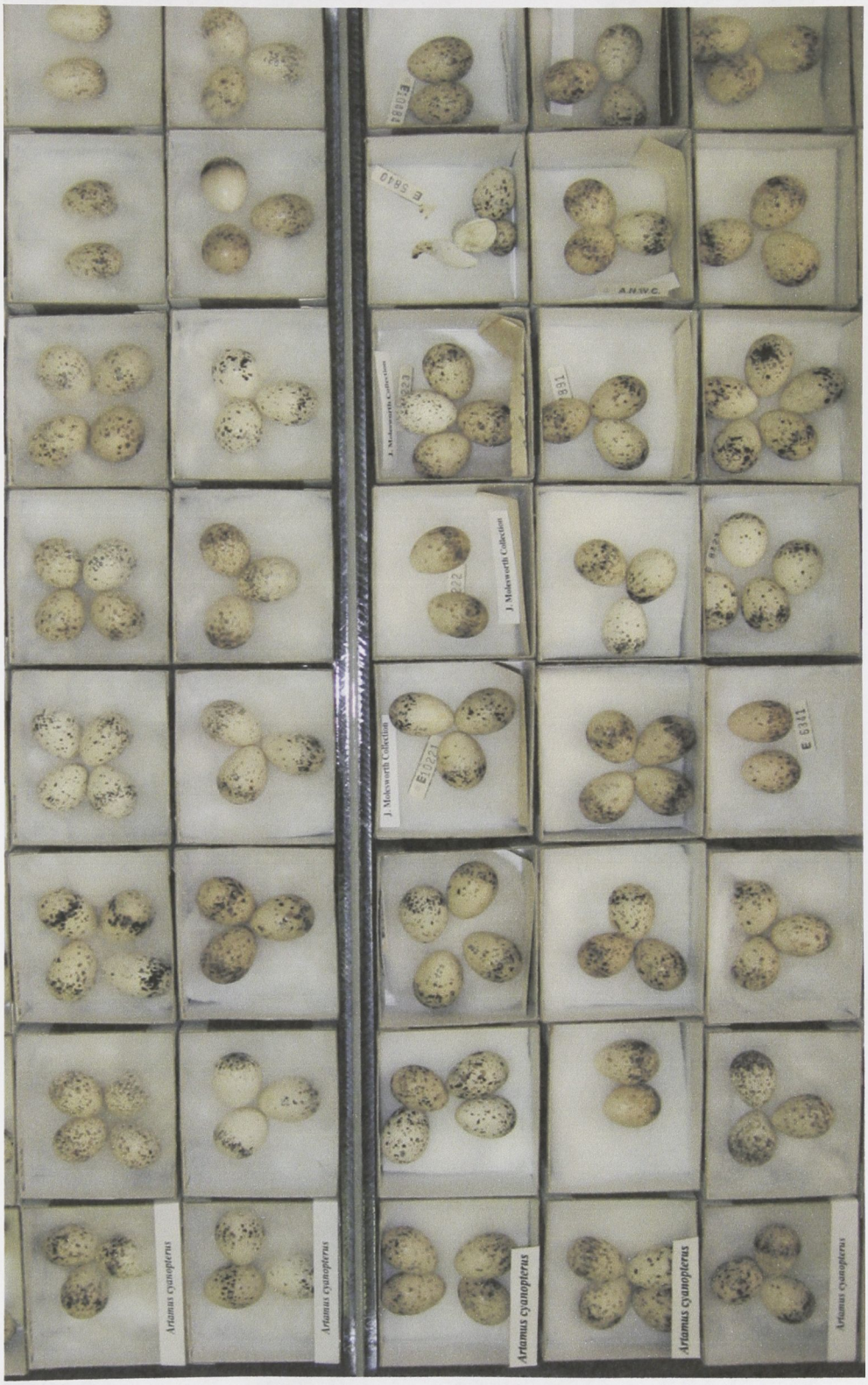
Yellow-tufted honeyeater

Locality	Reg. No.	Museum	Clutch Size	Age (years)
C1	5038	CSIRO	2	93
C1	5039	CSIRO	2	48
C1	50319	AM	2	98
C1	50320	AM	2	94
C1	55422	AM	2	98
M1	12793	CSIRO	2	30
M1	12794	CSIRO	2	30
M1	12795	CSIRO	2	30
M1	13322	CSIRO	2	29
M1	13323	CSIRO	2	29
M1	13324	CSIRO	2	29
M2	5025	CSIRO	2	48
M3	15187	CSIRO	2	87
M4	14529	CSIRO	2	47
M5	5021	CSIRO	2	72
M5	5024	CSIRO	2	32
M5	5026	CSIRO	2	67
M6	9898	CSIRO	3	101
M6	9899	CSIRO	2	105
M6	9900	CSIRO	2	101
M6	9902	CSIRO	2	101
M6	9903	CSIRO	2	101
M6	9904	CSIRO	2	101
M6	9905	CSIRO	2	101
M6	9906	CSIRO	2	101

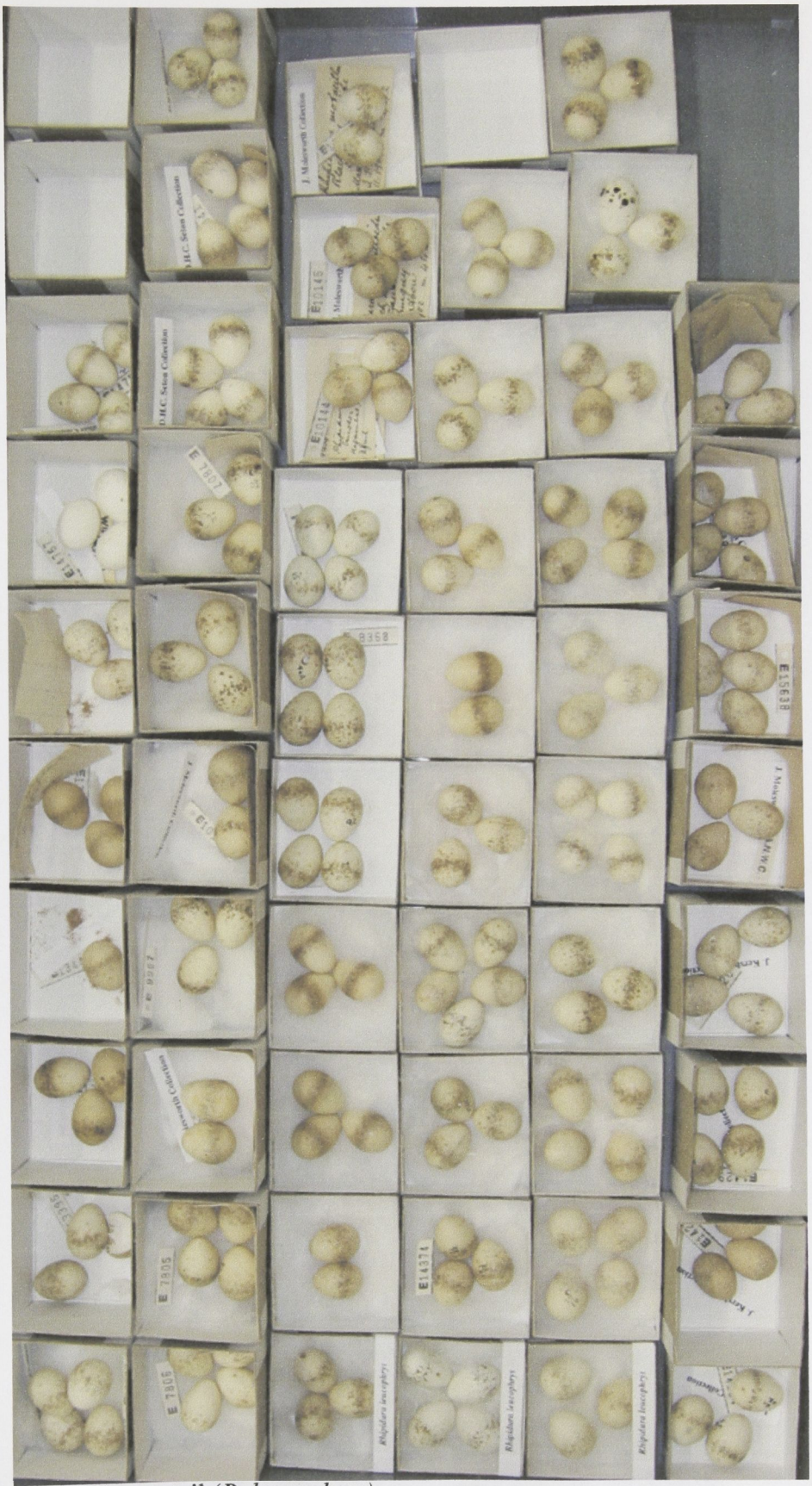
AM = Australian Museum
WA = Western Australian Museum
CSIRO = CSIRO, Canberra

APPENDIX B:

Plate 2. Examples of preserved clutches of each species from CSIRO collections



2a. Dusky woodswallow (*A. cyanopterus*)



2b. Willie wagtail (*R. leucophrys)*

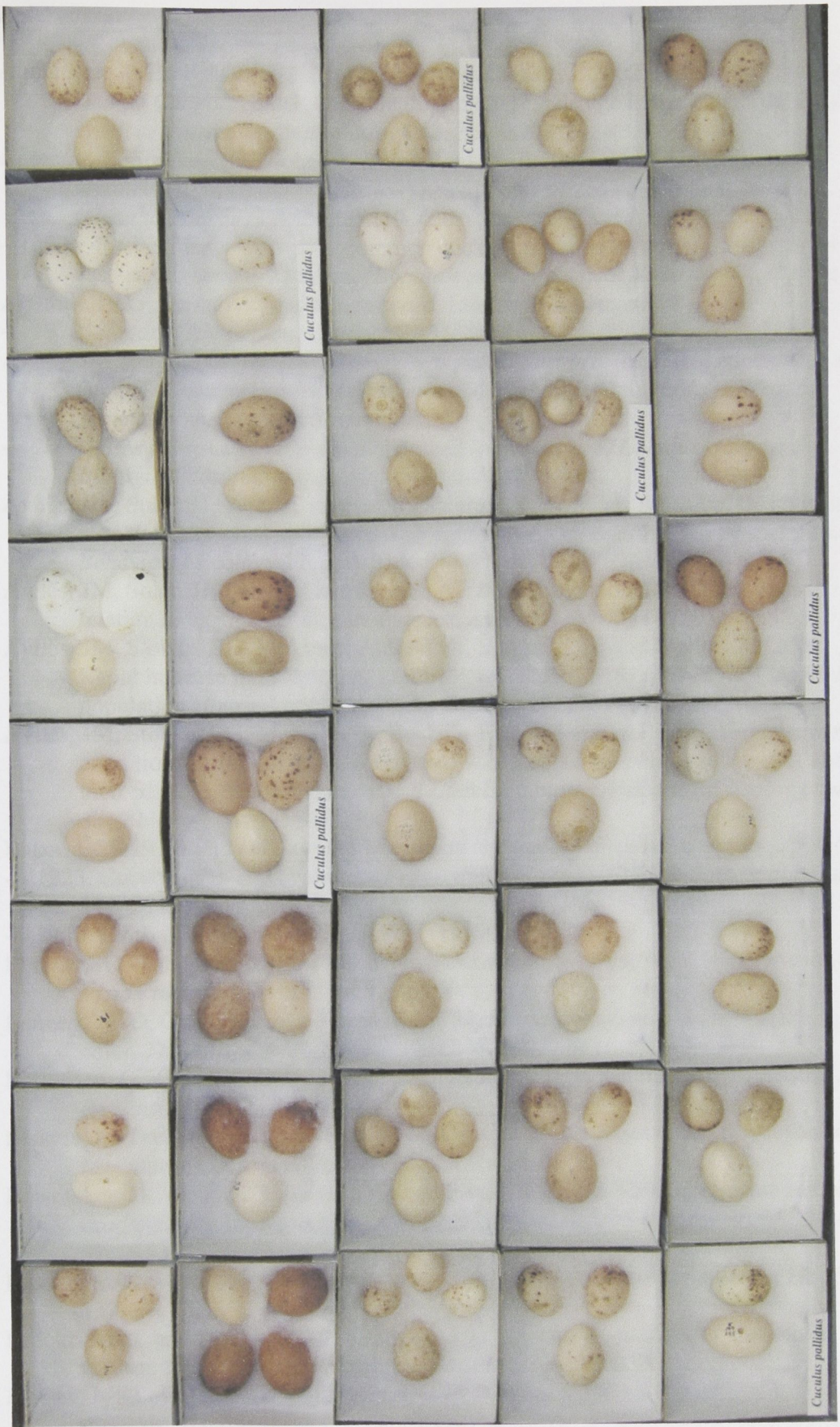
2c. White-plumed honeyeater (*L. penicillatus*)

2d. Red wattlebird (*A. carunculata*)

2e. Singing honeyeater (*L. virescens*)

2f. Yellow-throated miner (*M. flavigula*)

2g. Yellow-tufted honeyeater (*L. melanops*)



2h. Pallid cuckoo (*C. pallidus*)

References:

Amundsen, T, Brobakken, PT, Moksnes, A and Røskaft, E 2002. Rejection of common cuckoo *Cuculus canorus* eggs in relation to female age in the bluethroat *Luscinia svecica*. *Journal of Avian Biology* **33**: 366–370.

Antonov, A, Stokke, BG, Moksnes, A and Røskaft, E 2006. Egg rejection in marsh warblers (*Acrocephalus palustris*) heavily parasitized by common cuckoos (*Cuculus canorus*). *Auk* **123**: 419–430.

--- 2006. First evidence of regular common cuckoo, *Cuculus canorus*, parasitism on eastern olivaceous warblers, *Hippolais pallida elaeica*. *Naturwissenschaften*.

Aristotle 1936. *On marvellous things heard*. London: Heinemann.

--- 1970. *Historia Animalium*. London: W. Heinemann.

Avilés, JM and Møller, AP 2003. Meadow pipit (*Anthus pratensis*) egg appearance in cuckoo (*Cuculus canorus*) sympatric and allopatric populations. *Biological Journal of the Linnean Society* **79**: 543–549.

--- 2004. How is host egg mimicry maintained in the cuckoo (*Cuculus canorus*)? *Biological Journal of the Linnean Society* **82**: 57–68.

Avilés, JM, Soler, JJ, Pérez-Contreras, T, Soler, M and Møller, AP 2006. Ultraviolet reflectance of great spotted cuckoo eggs and egg discrimination by magpies. *Behavioral Ecology* **17**: 310–314.

Avilés, JM, Soler, JJ, Soler, M and Møller, AP 2004. Rejection of parasitic eggs in relation to egg appearance in magpies. *Animal Behaviour* **67**: 951–958.

Avilés, JM, Stokke, BG, Moksnes, A, Røskaft, E, Åsmul, M and Møller, AP 2006. Rapid increase in cuckoo egg matching in a recently parasitized reed warbler population. *Journal of Evolutionary Biology* **19**: 1901–1910.

Avilés, JM, Stokke, BG, Moksnes, A, Røskaft, E and Møller, AP 2007. Environmental conditions influence egg color of reed warblers *Acrocephalus scirpaceus* and their parasite, the common cuckoo *Cuculus canorus*. *Behavioral Ecology and Sociobiology* **61**: 475–485.

Baker, ECS 1913. The evolution of adaptation in parasitic cuckoos' eggs. *Ibis*: 384–398.

--- 1923. Cuckoo eggs and evolution. *Proceedings of the Zoological Society of London*: 277–294.

--- 1942. *Cuckoo Problems*. London: Witherby.

Bennett, ATD and Cuthill, IC 1994. Ultraviolet vision in birds: What is its function? *Vision Research* **34**: 1471–1478.

Bennett, ATD, Cuthill, IC and Norris, KJ 1994. Sexual selection and the mismeasure of color. *The American Naturalist* **144**: 848–860.

Beruldsen, G 2003. *Australian Birds their Nests and Eggs*. Kenmore Hills, Australia: G. Beruldsen.

Blakers, M, Davies, SJJF and Reilly, PN 1984. *The Atlas of Australian Birds*. Carlton: Melbourne University Press.

Bowmaker, JK, Heath, LA, Wilkie, SE and Hunt, DM 1997. Visual pigments and oil droplets from six classes of photoreceptor in the retinas of birds. *Vision Research* **37**: 2183–2194.

Brooke, MDL 2000. Why museums matter. *Trends in Ecology and Evolution* **15**: 136–137.

Brooke, MDL and Davies, NB 1988. Egg mimicry by cuckoos *Cuculus canorus* in relation to discrimination by hosts. *Nature* **335**: 630–632.

Brooke, MDL, Davies, NB and Noble, DG 1998. Rapid decline of host defences in response to reduced cuckoo parasitism: behavioural flexibility of reed warblers

in a changing world. *Proceedings of the Royal Society of London. Series B, Biological Sciences* **265**: 1277–1282.
--- 1998. Rapid decline of host defenses in response to reduced cuckoo parasitism: Behavioural flexibility of reed warblers in a changing world. *Proceedings of the Royal Society of London. Series B, Biological Sciences* **265**: 1277-1282.
Brooker, LC and Brooker, MG. 2005. Australian Cuckoo-Host Database. from http://www.users.bigpond.com/LesMikeBrooker/database.htm. 2007
Brooker, MG and Brooker, LC 1989. Cuckoo hosts in Australia. *Australian Zoological Review* **2**: 1–67.
--- 1991. Eggshell strength in cuckoos and cowbirds. *Ibis* **133**: 406–413.
Burkhardt, D and Maier, EJ 1989. The spectral sensitivity of a passerine bird is highest in the UV. *Naturwissenschaften* **76**: 82–83.
Chance, EP 1922. *The Cuckoo's Secret*. London: Sidgwick and Jackson.
--- 1940. *The Truth About the Cuckoo*. London: Country Life.
Chen, DM and Goldsmith, TH 1986. Four spectral classes of cone in the retinas of birds. *Journal of Comparative Physiology. A, Sensory, Neural, and Behavioral Physiology* **159**: 473–479.
Cherry, MI and Bennett, ATD 2001. Egg colour matching in an African cuckoo, as revealed by ultraviolet-visible reflectance spectrophotometry. *Proceedings of the Royal Society of London. Series B, Biological Sciences* **268**: 565–571.
Cherry, MI, Bennett, ATD and Cuthill, IC 1998. Matching of eggs of the Red-chested Cuckoo and its hosts: The role of the UV waveband. *Ostrich* **69**: 231.
Cherry, MI, Bennett, ATD and Moskát, C 2007. Host intra-clutch variation, cuckoo egg matching and egg rejection by great reed warblers. *Naturwissenschaften*.
Collias, EC 1993. Inheritance of egg-color polymorphism in the village weaver (*Ploceus cucullatus*). *Auk* **110**: 683–692.
Cuthill, IC and Bennett, ATD 1993. Mimicry and the eye of the beholder. *Proceedings of the Royal Society of London. Series B, Biological Sciences* **253**: 203–204.
Cuthill, IC, Bennett, ATD, Partridge, JC and Maier, EJ 1999. Plumage reflectance and the objective assessment of avian sexual dichromatism. *The American Naturalist* **160**: 183–200.
Cuthill, IC, Partridge, JC, Bennett, ATD, Church, SC, Hart, NS and Hunt, S 2000. Ultraviolet vision in birds. *Advances in the Study of Behavior* **29**: 159–214.
Dalton, R 2004. True colours. *Nature* **428**: 596–597.
Davies, NB 2000. *Cuckoos, Cowbirds and Other Cheats*. London: T & AD Poyser.
Davies, NB and Brooke, MDL 1988. Cuckoos versus reed warblers: adaptations and counter adaptations. *Animal Behaviour* **36**: 262–284.
--- 1989a. An experimental study of co-evolution between the cuckoo, *Cuculus canorus*, and its hosts. I. Host egg discrimination. *The Journal of Animal Ecology* **58**: 207–224.
--- 1989b. An experimental study of co-evolution between the cuckoo, *Cuculus canorus*, and its hosts. II. Host egg markings, chick discrimination and general discussion. *The Journal of Animal Ecology* **58**: 225–236.
Davies, NB, Brooke, MDL and Kacelnik, A 1996. Recognition errors and probability of parasitism determine whether reed warblers should accept or reject mimetic cuckoo eggs. *Proceedings of the Royal Society of London. Series B, Biological Sciences* **263**: 925–931.
Dawkins, R and Krebs, JR 1979. Arms race between and within species. *Proceedings of the Royal Society of London. Series B, Biological Sciences* **205**: 489–511.

Dittrich, W, Gilbert, F, Green, P, Mcgregor, P and Grewcock, D 1993. Imperfect mimicry: A pidgeon's perspective. *Proceedings of the Royal Society of London. Series B, Biological Sciences* **251**: 195–200.

Duckworth, JW 1991. Responses of breeding Reed Warblers Acrocephalus scirpaceus to mounts of Sparrowhawk *Accipiter nisus*, Cuckoo, *Cuculus canorus*, and Jay *Garrulus glandarius*. *Ibis* **133**: 68–74.

Endler, JA 1978. A predator's view of animal color patterns. *Evolutionary Biology* **11**: 319–364.

--- 1990. On the measurement and classification of colour in studies of animal colour patterns. *Biological Journal of the Linnean Society* **41**: 315–352.

Freeman, S 1988. Egg variability and conspecific nest parasitism in the *Ploceus* weaverbirds. *Ostrich* **59**: 49–53.

George, LT, Fowler, AC, Knight, RL and McEwen, LC 1992. Impacts of a severe drought on grassland birds in western North Dakota. *Ecological Applications* **2**: 275–284.

Gibbs, HL, Sorenson, MD, Marchetti, K, Brooke, MDL, Davies, NB and Nakamura, H 2000. Genetic evidence for female host-specific races of the common cuckoo. *Nature* **407**: 183–186.

Goldsmith, TH 1990. Optimization, constraint, and history in the evolution of eyes. *The Quarterly Review of Biology* **65**: 1990.

Grim, T 2005. Mimicry vs. similarity: which resemblances between brood parasites and their hosts are mimetic and which are not? *Biological Journal of the Linnean Society* **84**: 69–78.

Hart, NS and Hunt, DM 2007. Avian visual pigments: Characteristics, spectral tuning, and evolution. *The American Naturalist* **169**: S7–S26.

Hauber, ME, Moskát, C and Bán, M 2006. Experimental shift in hosts' acceptance threshold of inaccurate-mimic brood parasite eggs. *Biology Letters* **2**: 177–180.

Higgins, PJ, Ed. 1999. *Handbook of Australian, New Zealand and Antarctic Birds*. Parrots to Dollarbird. Melbourne, Australia: Oxford University Press.

Higgins, PJ, Peter, JM and Cowling, SJ, Eds. 2006. *Handbook of Australian, New Zealand and Antarctic Birds*. Boatbill to Starlings. Melbourne, Australia: Oxford University Press.

Higgins, PJ, Peter, JM and Steele, WK, Eds. 2001. *Handbook of Australian, New Zealand and Antarctic Birds*. Tyrant-Flycatchers to Chats. Melbourne, Australia: Oxford University Press.

Higuchi, H 1989. Responses of the bush warbler, *Cettia diphone*, to artificial eggs of *Cuculus canorus* in Japan. *Ibis* **131**: 94–98.

Hill, DP and Sealy, SG 1994. Desertion of nests parasitized by cowbirds: have clay-coloured sparrows evolved an anti-parasite defence? . *Animal Behaviour* **48**: 1063–1070.

Honza, M, Procházka, P, Stokke, BG, Moksnes, A, Røskaft, E, Čapek, M, Jr. and Mrlík, V 2004. Are blackcaps current winners in the evolutionary struggle against the common cuckoo? *Journal of Ethology* **22**: 175–180.

Hunt, S, Kilner, RM, Langmore, NE and Bennett, ATD 2003. Conspicuous, ultraviolet-rich mouth colours in begging chicks. *Proceedings of the Royal Society of London. Series B, Biological Sciences* **270**: S25–S28.

Huth, HH and Burkhardt, D 1972. Der spektrale Sehbereich eines Violettohr-Kolibris. *Naturwissenschaften* **59**: 650.

Jackson, WM 1998. Egg discrimination and egg-color variability. In: *Parasitic birds and their hosts*. Ed. by S. I. Rothstein and S. K. Robinson. New York: Oxford University Press**:** 407–416.

Janzen, DH 1980. When it is coevolution? *Evolution* **34**: 611–612.
Jenner, E 1788. Observations on the natural history of the cuckoo. *Philosophical Transactions of the Royal Society of London* **78**: 219–237.
Jones, J, Doran, PJ and Holmes, RT 2003. Climate and food synchronize regional forest bird abundances. *Ecology* **84**: 3024–3032.
Jourdain, FCR 1925. A study of parasitism in the cuckoos. *Proceedings of the Zoological Society of London*: 639–667.
Karcza, Z, Moskát, C, Cherry, MI and Kisbenedek, T 2003. Experimental Manipulation of Intraclutch Variation in the Great Reed Warbler Shows No Effect on Rejection of Parasitic Eggs. *Ethology* **109**: 15–22.
Kelber, AM, Vorobyev and Osorio, D 2003. Animal colour vision: behavioural tests and physiological concepts. *Biological Reviews* **78**: 81–118.
Kilner, RM 2006. The evolution of egg colour and patterning in birds. *Biological Reviews* **81**: 383–406.
Krüger, O and Davies, NB 2004. The evolution of egg size in the brood parasitic cuckoos. *Behavioral Ecology* **15**: 210–218.
Kuiper, SM and Cherry, MI 2002. Brood parasitism and egg matching in the Red-chested Cuckoo *Cuculus solitarius* in southern Africa. *Ibis* **144**: 632–639.
Lack, D 1968. *Ecological adaptations for breeding in birds*. London: Methuen.
Lahti, DC 2005. Evolution of bird eggs in the absence of cuckoo parasitism. *Proceedings of the National Academy of Sciences of the United States of America* **102**: 18057–18062.
Lahti, DC and Lahti, AR 2002. How precise is egg discrimination in weaverbirds? *Animal Behaviour* **63**: 1135–1142.
Langmore, NE, Hunt, S and Kilner, RM 2003. Escalation of a coevolutionary arms race through host rejection of brood parasitic young. *Nature* **422**: 157–160.
Langmore, NE, Kilner, RM, Butchart, SHM, Maurer, G, Davies, NB, Cockburn, A, Macgregor, NA, Peters, A, Magrath, MJL and Dowling, DK 2005. The evolution of egg rejection by cuckoo hosts in Australia and Europe. *Behavioral Ecology* **16**: 686–692.
Lawes, MJ and Kirkman, S 1996. Egg recognition and interspecific brood parasitism rates in red bishops. *Animal Behaviour* **52**: 553–563
Lindholm, AK and Thomas, RJ 2000. Differences between populations of reed warblers in defences against brood parasitism. *Behaviour* **137**: 25–42.
Lotem, A, Nakamura, H and Zahavi, A 1992. Rejection of cuckoo eggs in relation to host age: a possible evolutionary equilibrium. *Behavioral Ecology* **3**: 128–132.
--- 1995. Constraints on egg discrimination and cuckoo-host co-evolution. *Animal Behaviour* **49**: 1185–1209.
Lovászi, P and Moskát, C 2004. Break-down of arms race between the red-backed shrike (*Lanius collurio*) and common cuckoo (*Cuculus canorus*). *Behaviour* **141**: 245–262.
Maier, EJ 1992. sensitivities including the UV of the passeriform bird *Leiothrix lutea*. *Journal of Comparative Physiology. A, Sensory, Neural, and Behavioral Physiology* **170**: 709–714.
Marchetti, K 2000. Egg rejection in a passerine bird: size does matter. *Animal Behaviour* **59**: 877–883.
Moksnes, A, Røskaft, E and Braa, AT 1991. Rejection behaviour by common cuckoo hosts towards artificial brood parasite egg. *Auk* **108**: 348–354.
Moksnes, A, Røskaft, E, Braa, AT, Korsnes, L, Lampe, H and Pedersen, HC 1990. Behavioural responses of potential hosts towards artificial cuckoo eggs and dummies. *Behaviour* **116**: 64–89.

Moksnes, A, Røskaft, E and Korsnes, L 1993. Rejection of cuckoo (*Cuculus canorus*) eggs by meadow pipits (*Anthus pratensis*). *Behavioral Ecology* **4**: 120-127.

Møller, AP and Petrie, M 1991. *Evolution of intraspecific variability in birds' eggs: is intraspecific nest parasitism the selective agent?* Christchurch: Hutcheson, Bowman and Stewart Ltd.

Molnár, B 1944. The cuckoo in the Hungarian plain. *Aquila* **51**: 100–112.

Moskát, C, Szentpéteri, J and Barta, Z 2002. Adaptations by great reed warblers to brood parasitism: a comparison of populations in sympatry and allopatry with the common cuckoo. *Behaviour* **139**: 1313–1329.

Øien, IJ, Moksnes, A and Røskaft, E 1995. Evolution of variation in egg color and marking pattern in European passerines: adaptations in a evolutionary arms race with the cuckoo, Cuculus canorus. *Behavioral Ecology* **6**: 166–174.

Payne, RB 1967. Interspecific communication signals in parasitic birds. *The American Naturalist* **101**: 363–375.

--- 1974. The evolution of clutch size and reproductive rates in parasitic cuckoos. *Evolution* **28**: 169–181.

--- 1977. The ecology of brood parasitism in birds. *Annual Review of Ecology and Systematics* **8**: 1–28.

Polačiková, L, Honza, M, Procházka, P, Topercer, J and Stokke, BG 2007. Colour characteristics of the blunt egg pole: cues for recognition of parasitic eggs as revealed by reflectance spectrophotometry. *Animal Behaviour* **74**: 419–427.

Procházka, P and Honza, M 2003. Do Common Whitethroats (*Sylvia communis*) discriminate against alien eggs? *Journal für Ornithologie* **144**: 354–363.

--- 2004. Egg discrimination in the yellowhammer. *Condor* **106**: 405–410.

Rahn, H and Ar, A 1974. The avian egg: incubation time and water loss. *Condor* **76**: 147–152.

Røskaft, E, Moksnes, A, Meilvang, D, Bicík, V, Jemeliková, J and Honza, M 2002. No evidence for recognition errors in *Acrocephalus* warblers. *Journal of Avian Biology* **33**: 31–38.

Røskaft, E, Moksnes, A, Stokke, BG, Bicík, V and Moskát, C 2002. Aggression to dummy cuckoos by potential European cuckoo hosts. *Behaviour* **139**: 613–628.

Rothstein, SI 1975. Evolutionary rates and host defenses against avian brood parasitism. *The American Naturalist* **109**: 161–176.

--- 1975. An experimental and telenomic investigation of avian brood parasitism. *Condor* **77**: 250–271.

--- 1975. Mechanisms of avian egg-recognition: do birds know their own eggs? *Animal Behaviour* **23**: 268–278.

--- 1990. A model system for coevolution: avian brood parasitism. *Annual Review of Ecology and Systematics* **21**: 481–508.

Rothstein, SI, Patten, MA and Fleischer, RC 2002. Phylogeny, specialization, and brood parasite–host coevolution: some possible pitfalls of parsimony. *Behavioral Ecology* **13**: 1–10.

Schodde, R and Mason, IJ 1999. *Directory of Australian Birds: Passerines*. Melbourne, Victoria, Australia: CSIRO Publishing.

Sealy, SG 1995. Burial of cowbird eggs by parasitized yellow warblers: an empirical and experimental study. *Animal Behaviour* **49**: 877–889.

Seel, DC 1973. Egg-laying in the cuckoo. *British Birds* **66**: 528–535.

Soler, JJ, Aviles, JM, Soler, M and Møller, AP 2003. Evolution of host egg mimicry in a brood parasite, the great spotted cuckoo. *Biological Journal of the Linnean Society* **79**: 551–563.

Soler, JJ and Møller, AP 1996. A comparative analysis of the evolution of variation in appearance of eggs of European passerines in relation to brood parasitism. *Behavioral Ecology* **7**: 89–94.

Soler, JJ, Soler, M and Møller, AP 2000. Host recognition of parasite eggs and the physical appearance of host eggs: the Magpie and its brood parasite the Great Spotted Cuckoo. *Etología* **8**: 9–16.

Soler, M and Møller, AP 1990. Duration of sympatry and coevolution between the great spotted cuckoo and its magpie host. *Nature* **343**: 748–750.

Solomon, SE 1987. Egg shell pigmentation. In: *Egg quality - current problems and recent advances*. Ed. by R. G. Wells and C. G. Belyavin. Kent, England: Butterworth & Co. Ltd. **20:** 147–157.

Spaw, CD and Rohwer, S 1987. A comparative study of eggshell thickness in cowbirds and other passerines. *Condor* **89**: 307–318.

Starling, M 2005. The Effect of Interspecific Interactions on the Breeding Behaviour of the Willie Wagtail, *Rhipidura leucophrys*. *Division of Botany and Zoology*. Canberra: The Australian National University, *Bachelor of Science (Honours)*.

Starling, M, Heinsohn, R, Cockburn, A and Langmore, NE 2006. Cryptic gentes revealed in pallid cuckoos *Cuculus pallidus* using reflectance spectrophotometry. *Proceedings of the Royal Society of London. Series B, Biological Sciences* **273**: 1929–1934.

Stokke, BG, Moksnes, A and Røskaft, E 2002. Obligate brood parasites as selective agents for evolution of egg appearance in passerine birds. *Evolution* **56**: 199–205.

--- 2005. The enigma of imperfect adaptations in hosts of avian brood parasites. *Ornithological Science* **4**: 17–29.

Stokke, BG, Moksnes, A, Røskaft, E, Rudolfsen, G and Honza, M 1999. Rejection of artificial cuckoo (*Cuculus canorus*) eggs in relation to variation in egg appearance among reed warblers (*Acrocephalus scirpaceus*). *Proceedings of the Royal Society of London. Series B, Biological Sciences* **266**: 1483–1488.

Stokke, BG, Rudolfsen, G, Moksnes, A and Røskaft, E 2004. Rejection of conspecific eggs in chaffinches: the effect of age and clutch characteristics. *Ethology* **110**: 459–470.

Swynnerton, CFM 1918. Rejections by birds of eggs unlike their own: with remarks on some of the cuckoo problems. *Ibis*: 127–154.

Takasu, F 2003. Co-evolutionary dynamics of egg appearance in avian brood parasitism. *Evolutionary Ecology Research* **5**: 345–362.

Taylor, M 1992. *Birds of the Australian Capital Territory, an Atlas*. Canberra: Canberra Ornithologists Group and National Capital Planning Authority.

Underwood, TJ and Sealy, SG 2002. Adaptive significance of egg coloration. In: *Avian incubation- behaviour, environment, and evolution*. Ed. by D. C. Deeming. Oxford: Oxford University Press**:** 280–298.

Victoria, JK 1972. Clutch characteristics and egg discriminative ability of the African village weaverbird *Ploceus cucullatus*. *Ibis* **114**: 367–376.

Welbergen, J, Komdeur, J, Kats, R and Berg, M 2001. Egg discrimination in the Australian reed warbler (*Acrocephalus australis*): rejection response toward model and conspecific eggs depending on timing and mode of artificial parasitism. *Behavioral Ecology* **12**: 8-15.

Wright, AA 1972. The influence of ultraviolet radiation on the pigeon's color discrimination. *Journal of the Experimental Analysis of Behavior* **17**: 325–337.

Wyllie, I 1981. *The Cuckoo*. London: Batsford.